

THE EFFECTS OF OAT β -GLUCAN CONSUMPTION
ON THE ENERGY INTAKES OF HEALTHY
INDIVIDUALS

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“Being a PhD student is like becoming all of the Seven Dwarves. In the beginning you’re Dopey and Bashful. In the middle, you are usually sick (Sneezy), tired (Sleepy), and irritable (Grumpy). But at the end, they call you Doc, and then you’re Happy.”

- Ronald T. Azuma

This PhD thesis is dedicated to everyone who has convinced me to believe in myself to overcome the obstacles I’ve faced during this doctoral rollercoaster, and most importantly, to those who have encouraged me to keep running the ‘marathon’, even if I had to walk during a few phases.

For Mary. M. Paterson (†) Peter Paterson (†), and Graeme Morrison (†) who I miss dearly.

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Abstract

Overweight and obesity are disease states of a huge public health concern, therefore strategies to impede or reverse the current detrimental overweight and obesity epidemic are of fundamental importance. It is important to understand dietary factors that affect appetite and food intake both in short- and long-term, as energy intake can lead to positive energy balance.

Following the discovery of the bioactivity of cereal soluble fibre, (1→3,1→4)- β -D-glucan, there has been extensive attention among researchers, the food industry and consumers since the 1980s. Several authorities, including the U.S Food and Drug Administration (FDA) and European Food and Safety Administration (EFSA) have acknowledged the cardiovascular (CV) health benefits of β -glucan consumption by broadcasting ratified health claims based on robust scientific evidence. Yet despite evidence to suggest that cereal β -glucan can beneficially impact on appetite, the underpinning mechanisms whereby β -glucan influences energy intakes remain elusive.

Given that there is no ratified health claim for β -glucan consumption and satiety, the aim of this work was to investigate the impact of oat β -glucan consumption on energy intakes of healthy individuals over both short- and medium-term.

β -glucan enrichment of a semi-solid, viscous breakfast (4 g oat β -glucan) had no effect on subsequent eating ($p=0.388$) in 33 normal-weight subjects (22 female/11 male, mean age (y): 27.0 ± 1.0 , BMI (kg/m^2): 23.5 ± 0.4), however there was a significant increase in subjective feelings of satiety ($p=0.034$) and fullness ($p=0.048$). Additionally, attenuation of glucose ($p<0.001$) and insulin ($p=0.001$) were reported alongside a decreased response in GLP-1 after 90 minutes ($p=0.021$) in study A.

A novel β -glucan-enriched oatcake snack (4.46 g β -glucan) had no effect on daily energy intakes of healthy overweight and obese subjects (11 female/2 male, mean age (y): 34 ± 9 , BMI (kg/m^2): 29.8 ± 4.4) when consumed daily for six weeks when compared to a control snack group during week 3 ($p=0.39$) or week 6 ($p=0.58$) of the study. Moreover, there were no significant improvements in markers of abdominal obesity, waist circumference (WC, $p=0.67$), sagittal abdominal diameter (SAD, $p=0.38$), BMI ($p=0.99$) or body fat percentage (BF%, $p=0.54$) between groups in study B following 6 weeks of β -glucan-enriched snack consumption.

To conclude, evidence reported in this thesis supports evidence that oat β -glucan consumption does not influence short- or medium-term energy intakes in healthy individuals, however, in the short term β -glucan does increase subjective ratings of appetite and attenuates postprandial glucose, insulin and GLP-1 responses.

Keywords: oat β -glucan, energy intake, appetite, GLP-1, body composition

Abbreviations

%CV	Coefficient of variation
ADP	Air displacement plethysmography
AgRP	Agouti-related protein
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ARC	Arcuate nucleus
AUC	Area under the curve
BBB	Blood-brain-barrier
BF%	Body fat percentage
BIA	Bioelectrical impedance analysis
BMI	Body mass index
bpm	Beats per minute
BMRest	Estimation of basal metabolic rate
BP	Blood pressure
CART	Cocaine- and amphetamine-regulated transcript
CCK	Cholecystokinin
CHO	Carbohydrate
CNS	Central nervous system
CT	Computerised tomography
CV	Cardiovascular
CVD	Cardiovascular disease
CVO	Circumventricular organ
DBP	Diastolic blood pressure
DEBQ	Dutch Eating Behaviour Questionnaire
DEXA	Dual energy X-ray absorptiometry
DPP-4	Dipeptidyl peptidase-4
DRV	Dietary reference value
EARS	Electronic appetite rating systems
EFSA	European Food and Safety Administration

EIrep	reported energy intake
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FFAR2	Free fatty acid receptor-2
FFAR3	Free fatty acid receptor-3
FFQ	Food frequency questionnaire
fMRI	Functional magnetic resonance imaging
G6PC	Glucose-6-phosphatase
GE	Gastric emptying
GER	Gastric emptying rate
GI	Gastrointestinal
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-like peptide-1
GLP-1R	GLP-1 receptor
GPR41	G-protein coupled receptor 41
GPR43	G-protein coupled receptor 43
GRPP	Glicentin related pancreatic polypeptide
HbA1c	Glycated haemoglobin (A1c)
HDL	High-density lipoprotein cholesterol
HMW	High molecular weight
HPV	Hepatic portal vein
IGN	Intestinal gluconeogenesis
ILSI	International Life Sciences Institute
ISAK	International Society for the Advancement of Kinanthropometry
IV	Intravenous
kcal	Calories
LDL	Low density lipoprotein
LMW	Low molecular weight
MetS	Metabolic syndrome
mPa.s	Millipascal seconds
mTOR	Mechanistic target of rapamycin

MW	Molecular weight
NDNS	National Diet & Nutrition Survey
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NPY	Neuropeptide Y
NSP	Non-starch polysaccharide
NTS	Nucleus tractus solitarius
OGTT	Oral glucose tolerance test
OXM	Oxyntomodulin
PAL	Physical activity level
PASSCLAIM	Process for the Assessment of Scientific Support for Claims on Foods
PGX	PolyGlycopleX®
PMS	Premenstrual syndrome
POMC	Proopiomelanocortin
PVN	Paraventricular nucleus
PYY	Peptide YY
QC	Quality control
QMU	Queen Margaret University
RTEC	Ready-to-eat cereal
SACN	Scientific Advisory Committee on Nutrition
SAD	Sagittal abdominal diameter
SAT	Subcutaneous adipose tissue
SBP	Systolic blood pressure
SCFA	Short chain fatty acid
SEM	Standard error of the mean
SFA	Saturated fatty acid
SHeS	Scottish Health Survey
SPAQ	Scottish Physical Activity Questionnaire
SSS	Sensory specific satiation
T2DM	Type 2 diabetes mellitus
TFA	Trans fatty acid

TG	Triglycerides
VAN	Vagal afferent neuron
VAS	Visual analogue scale
VAT	Visceral adipose tissue
WC	Waist circumference
WHO	World Health Organisation
XOS	Xylooligosaccharides

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Chapter 1

1.0 Introduction

“Sir, – Those of us who spent our professional lives in tropical Africa would like to express agreement with the suggestion of Dr. K. W. Heaton (Dec. 22, p. 1418) that food fibre has a preventable role in preventing obesity... Fibre is an important constituent of the normal diet. But neither the word fibre or unavailable carbohydrate receives much discussion in any book on obesity.”

- Trowell, H. Lancet 1974.

Overweight and obesity are terms used to define abnormal or excessive fat accumulation that may impair an individual's health status. The most recent Scottish Health Survey (SHeS) in 2017 reports that 65% of Scottish adults are overweight, obese or morbidly obese (SHeS 2017). Although this figure has plateaued since 2008, overweight and obesity still remains a public health epidemic, and highlights that Government policy initiatives implemented such as *Preventing Overweight and Obesity in Scotland: A Route Map Towards Healthy Weight* (Scottish Government 2010) has not improved Scotland's obesity prevalence.

Given their preventable nature, overweight and obesity are disease states which cause a huge public health concern, therefore strategies to impede or reverse the current detrimental overweight and obesity trends are of fundamental importance to Scotland from both an economic and health perspective. In most individuals with glucose intolerance or type 2 diabetes (T2DM), there are a multiple set of risk factors that commonly appear together, forming what is known as the 'Metabolic Syndrome' (MetS). This 'clustering' of metabolic abnormalities that occur in the same individual appear to present a substantial additional CV risk over and above the sum of the risk associated with each abnormality (Cloetens et al. 2012). Obesity contributes to hypertension, high serum cholesterol, low high-density lipoprotein cholesterol (HDL) and hyperglycaemia, and is individually associated with elevated cardiovascular disease (CVD) risk (Carey et al. 1997; Zimmet et al. 2001; Hu et al. 2004). The risk of severe health consequences has been shown to rise in relation to an increase in body mass index (BMI) (Lee et al. 1993), however it is an excess of body fat in the abdomen, measured easily by waist circumference (WC), which is more indicative of the MetS profile than BMI.

Obesity, therefore is a target for improving not only health but quality of life given its association with chronic disease development. It is essential to find dietary factors that have beneficial effects on appetite and food intake regulation. The regulation of appetite and food intake is, however, a complex system, influenced strongly by not only the food and its various qualities, but also a range of individual (internal) characteristics and hedonic, social and environmental (external) factors (Blundell et al. 2010; Langhans and Geary 2010). In this system, the external factors may markedly interfere with the internal homeostatic control. Even so, food is an integral part of these “outer” and “inner” systems and the attributes of foods that assist the bodily system towards energy balance should be recognized.

Conversely to Trowell (1974), the health benefits of dietary fibre have been well documented in the literature over the past few decades following on from his early observational studies. The characterization and analytical methods of dietary fibre are now more advanced, with focus on linking the structural features of dietary fibre components to their physiological functions. Currently, a clear definition of dietary fibre is generally agreed, and more emphasis is placed on a comprehensive understanding of its structure-function relationship and applications in functional food product development. There is no doubt that solid health claims of dietary fibre can be derived from a large amount of clinical data; however, the detailed mechanisms that can elucidate these beneficial effects are still under investigation, particularly with regards to energy intake, an area that is of key importance for tackling overweight and obesity.

Chapter 2

2.0 Literature Review

2.1 Evolution of the Western Diet

The evolutionary history of *hominins* has been characterized by significant dietary changes, which include the introduction of meat eating, cooking, and the changes associated with plant and animal domestication (Luca et al. 2010). Across millions of years the evolution of the human diet has been striking – from the very early pre-agricultural “table” of hunter-gatherers to the affluent buffets of post-agricultural Western societies (Cordain et al. 2005). The diet of modern man, composed of functional and processed foods, has changed considerably since the days of ancient foragers, who predominantly consumed minimally processed, wild plant and animal based foods. The introduction of agriculture and animal husbandry (~10,000 years ago), the Industrial Revolution (~200 years ago) (Cordain et al. 2005), together with the exceptional technological advances in food production, delivery and storage during recent decades, have been crucial in dietary evolution. In support of the evolutionary discordance theory, that is the connection between diet and “diseases of civilization”, it has been proposed that the modern-day diet has evolved too quickly and too distantly from the diet which our ancestors adapted to and survived with, which in turn conditioned our ancient genetic makeup and physiology (Eaton et al. 1988; Luca et al. 2010). Food staples and food-processing procedures introduced during the Neolithic and Industrial Periods have fundamentally altered crucial nutritional characteristics of ancestral *hominin* diets, including glycaemic load, fatty acid composition, macronutrient composition, micronutrient density, acid-base balance, sodium-potassium ratio and fibre content (Cordain et al. 2005). For example, the high intake of wild plant foods by ancestral humans necessarily provided a great deal of fibre. Proximate analyses of uncultivated vegetables and fruits consumed by hunter-gatherers show that they were substantially more fibrous (crude estimate of 133 g dietary fibre/kg) than are those now commercially available (42 g/kg). Based on a 50:50 animal–vegetable subsistence ratio, it is suggested that an ancestral average total dietary fibre intake was more than 100 g per

day (Eaton 2006). The Scientific Advisory Committee on Nutrition (SACN 2015) suggest that an adequate intake of total fibre from food should be 30 g per day for adults, a value which is considerably more than current UK fibre intakes for both males and females. Dietary fibre is not digested by digestive enzymes and passes through the gut to the large intestine where it is fermented by microflora. Fruits and vegetables contain fibre that is more completely fermentable than that found in cereals, a distinction that appears to influence the physiological and health effects related to fibre intake. Thus, fermentability of ancestral dietary fibre would have been much greater than that typically found in today's fibre-containing foods.

As well as the aforementioned characteristics, the physiochemical structure of foods also plays a key role in the nutritional characteristics of diets, and are often overlooked in understanding the metabolic responses and long-term health consequences of various diets (Chau et al. 2004). For example, different types of food products with the same nutritional profile, all containing soluble fibre oat β -glucan but processed by different techniques (granola, muffins, bread, porridge and pasta) have been shown to elicit different effects on the glycaemic responses of overweight adults (Regand et al. 2009), highlighting that the physiochemical nature of the food matrix itself determines functionality more than the actual nutritional content.

Despite the changes in several characteristics of staple foods, which resulted from industrial affluence and modern food technology, the development of food science and food industry has unquestionably benefited nutrition and overall health and wellbeing in man (Eaton 2006). Technological and scientific advances in food production and processing, distribution and storage have radically improved food safety, alongside food availability and affordability of a vast range of foods in developed societies. As a result, it can be suggested that these achievements are much more important contributors to increasing life expectancy than nutritional advances relative to chronic disease prevention could ever be (Eaton et al. 2002).

Of course, the advances seen in industrialized food production also has its disadvantages. There is a constant variety of refined, energy dense, affordable and highly palatable food products manufactured and intensively advertised by modern food industry. Consequently, there exists a gradual undermining of health benefits achieved by improved food safety and availability, of which corrective actions are urgently

required (Swinburn et al. 2011). Despite public health promotion, the incidence of non-communicable diseases of adulthood, such as MetS, CVD and T2DM, continue to rise in the Western world and westernizing populations. Even though it is neither applicable nor conceivable to revert back to the diet of our early ancestors, it may be beneficial to utilize the health-supporting characteristics of these early diets, i.e., food and food ingredients from early ancestral diets that have been demonstrated to possess health benefits, even in the form of tailored or functional foods (Jew et al. 2009; Lindeberg 2012). With the aid of modern food technology, this could be performed by modifying and supplementing the modern diet with favourable attributes. For instance, many studies have investigated the health benefits of various functional food ingredients, including omega-3 fatty acids, polyphenols, fibre and plant sterols. However, in order to create optimal health benefiting foods, it is paramount to gain a clear understanding of the way in which food will achieve these desired effects (Lentle and Janssen 2010). This requires detailed knowledge of gut physiology and the physicochemical properties of foods that influence physiology and efficiency of digestion and absorption both at the organ and the cellular level (Lentle and Janssen 2010). Ultimately, this process might involve reintroduction of the essential components from the diet and lifestyle of our early ancestors. The remodelling of the dietary elements may not affect our life expectancy profoundly, but instead affect good health years and lessen the burden of global public health care costs (Eaton et al. 1988; Jew et al. 2009; Kuipers et al. 2010).

2.2 Regulation of Food Intake

Humans enjoy diverse lifestyles and this complexity is reflected in their eating behaviours and habits. Although the stereotypical '*three meals a day*' may typify the frequency of many individuals' meal episodes, the volume and number of eating bouts, as well as the total amount of food eaten each day tends to be variable. Because eating is our major source of metabolic fuel and means of obtaining a number of essential nutrients, it is an integral part of homeostatic regulation.

2.2.1 Energy Homeostasis

The brain is a pivotal organ in the control of energy homeostasis (Schwartz et al. 2000; Flier 2004; Elmquist et al. 2005). The brain receives a continuous stream of diverse signals regarding energy status throughout the body and consequently influences energy consumption. In addition, the brain influences the entry of nutrients into the blood and their utilization by most tissues. It is responsible for integrating incoming information in the form of hormonal and neural signals regarding energetic needs or anticipated needs with environmental factors, such as location and timing of food, memories of past eating experiences and hedonic factors (Woods 2009).

A significant role of the brain is to ensure adequate circulating energy for immediate tissue needs as well as adequate stored energy to survive intervals when external energy is limited (Seeley and Woods 2003). Normally, the levels of energy-rich fuels in the blood (glucose, amino acids, lipids) are relatively constant, such as there is a balance between fuels sequestered by tissues and fuels secreted by the liver and adipocytes. An exception to this is when newly digested nutrients enter the blood (Friedman 1998) and afferent signals are relayed to the brain as a consequence of the influx of plasma glucose and nutrients detected. The ability to limit meal size is of particular importance to avoid large perturbations of plasma nutrients. Accurately anticipating meals and subsequently coordinating ongoing information about energy being consumed (via satiation signals), the levels of fuels already in the plasma (via direct sensing by specialized cells in the brain and elsewhere), and the amount of energy present in various storage depots (via adiposity signals) allows optimal levels of energy rich nutrients to be stored and circulate in the body (Benelam 2009).

Under normal circumstances, the supply of energy in the blood does not decrease to anywhere near the threshold necessary to trigger eating. Eating is in fact a relatively inefficient way to get energy into the blood quickly. Unless pure glucose is available, foods must be processed and digestion initiated by the stomach before passing to the intestine for further digestion, processing and nutrient absorption (Langhans and Geary 2010). Despite the hours that pass before nutrients are absorbed in the blood, the concept that

eating is triggered as a means to replenish diminishing fuel supplies has remained for several decades. The glucostatic theory was introduced by Mayer (1953), who hypothesized that eating was initiated when glucose levels and utilization by specific cells in the hypothalamus were reduced. Similarly, meals were hypothesized to end when glucose levels and/or availability were restored to adequate levels. Such a process would imply dangerously low levels of glucose prior to the initiation of each meal. Nowadays, it is generally recognized that this system is most likely activated to the point of initiating a meal only in extreme metabolic emergencies (Langhans 1996).

The difference between the concentration of glucose in arterial and venous blood (arteriovenous difference) is largely a reflection of the rate of glucose assimilation in the extrahepatic tissues, mainly in the muscles. Therefore, any change in arteriovenous blood should provide valuable information regarding the effects of various factors which influence glucose digestion and absorption (Haeckel et al. 2002). Follow up studies since Mayer's theory have confirmed correlations with arteriovenous differences in blood glucose, hunger ratings and food intake under some circumstances (van Itallie et al. 1953; Stunkard et al. 1955). Despite this, other research has failed to show relationships between arteriovenous differences in blood glucose and hunger ratings of food intake (Bernstein and Grossman 1956; van Itallie and Hashim 1960). With regards to exogenous glucose, other research where hunger ratings and food intake were measured following intravenous (IV) infusions of glucose have reported either consistent (Stunkard and Wolff 1956; Booth et al. 1970), or inconsistent results (Grinker et al. 1971; Woo et al. 1984) with a role for glucose in the onset of hunger. These findings emphasized the role of decreased glucose utilization or decreased intracellular glucose concentrations rather than the absolute level of blood glucose as the stimulus for meal initiation (Chaput and Tremblay 2009). A sensitive way to evaluate satiety is to determine the onset latency of the next meal when freely requested by subjects deprived of time cues. Postprandial transient blood glucose declines have been reported to be associated with meal requests in time-blinded subjects (Melanson et al. 1990a; Melanson et al. 1999b). The role of glucose in the control of food intake is therefore believed to be dynamic; a satiety factor and initiation signal.

Knowledge of the regulation of food intake is crucial to an understanding of body weight and obesity. Strictly speaking, we should refer to the control of food intake whose expression is modulated in the interests of the regulation of body weight. Food intake is controlled, body weight is regulated (Hopkins et al. 2016). However, this semantic distinction only serves to emphasize the importance of food intake.

Despite considerable fluctuations in our daily food intake, many of us can adjust to overall energy intake and energy expenditure, resulting in relatively stable body weight over longer periods of time. This mechanism is termed energy homeostasis, a dynamic regulatory process controlling both short-term and long-term energy balance in the body (Schwartz et al. 2000; Badman and Flier 2005; Murphy and Bloom 2006). Yet despite these intricate control mechanisms, there continues to be an escalating number of overweight and obese individuals worldwide (Finucane et al. 2011), indicating that homeostatic mechanisms no longer function adequately in our modern environment, which is heavily influenced by westernized dietary habits and lifestyle (Murphy and Bloom 2006; Zheng et al. 2009).

Day-to-day food intake, which consists of a series of discrete feeding episodes, involves the co-ordination of both homeostatic (e.g. energy need) and non-homeostatic feedback (e.g. food hedonics and environmental factors) (Blundell and Gillet 2001). The communication among numerous organs, predominantly the brain, gut, liver, adipose tissue, pancreas and muscle, is a fundamental mechanism underlying the regulation of appetite and food intake. However, the continuous communication between the two major players, the gut and the central nervous system (CNS), is considered the major axis in controlling short-term homeostasis. Enteroendocrine cells in the intestinal mucosa detect luminal content pre- and postprandially, which facilitates the release of cell-specific peptides responsible for controlling gastrointestinal (GI) functions such as motility, secretion and absorption (Cummings and Overduin 2007). Various “taste receptors” on the enteroendocrine cells are also suggested to be involved in GI peptide secretion, as demonstrated by Gerspach et al. (2011) and Steinert et al. (2012). This peptide-specific signalling mechanism reports the peripheral short-term energy status to the CNS via circulation and/or neural activation. In turn, the CNS responds to these stimuli and coordinates adaptive responses via negative

or positive feedback to the peripheral targets affecting ultimately energy intake and expenditure and body fat stores (Schwartz et al. 2000; Wynne et al. 2005). Thus, the gut-brain-axis is at the epicentre of appetite control and energy balance, with the endocrinological capacity of the GI tract playing a key role under the influence of the brain.

Peripheral signals involved in the regulation of food intake and energy balance operate in two different timeframes, as such they are categorized as short- and long-term operators (Badman and Flier 2005; Wren and Bloom 2007), and are referred to as episodic and tonic signalling, respectively. In general, the long-term signals such as leptin reflect the volume of adipose stored in the body, and regulate body weight over longer periods of time (Schwartz et al. 2000; Wynne et al. 2005). The short-term signals arise from the GI tract and encompass gut hormones such as ghrelin, cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), regulating not only appetite but also a wide range of intestinal and other vital physiological functions (Chaudhri et al. 2006). Despite the difference in the timeframes of their actions the two systems overlap considerably, the long-term system has control over the short-term (Schwartz et al. 2000; Morton et al. 2006). Nonetheless, studies indicate that the categorization between long and short-term signals is, to a certain extent, artificial since many of the regulators, such as orexigenic ghrelin and anorexigenic PYY, have been identified as regulators both short- and long-term energy homeostasis (Wren and Bloom 2007; Karra et al. 2009; Castaneda et al. 2010).

The integration of the tonic and episodic signals reflects the brain's recognition of the current dynamic state of energy stores and the oscillating fluctuations of nutrients derived from eating and detected by episodic signalling. This integration is materialized in a set of neural pathways and receptors that extend from the nucleus tractus solitarius (NTS) and area postrema in the hindbrain, through to the discrete hypothalamic nucleus in the basal forebrain (Baptista et al. 2005).

The episodic signals fluctuate in harmony with eating patterns. The largest group of these peptides provides signals for satiation (meal termination) and satiety (postmeal inhibition of eating). For example, CCK is released by enteroendocrine cells in the duodenum in response to fat and protein ingestion and influences

meal size through a central action to terminate eating. Additionally, GLP-1, which elevates after eating, plays a role in inhibiting eating in the postprandial period. The action of PYY (3–36) is facilitated by the ‘ileal brake’ phenomenon (van Avesaat et al. 2015). The gastric peptide ghrelin is a meal-related signal with an appetite-inducing effect because it is increased before meals and decreases after meals. One common feature of the action of these peptides is to adjust the rate of gastric emptying (GE), and this may play a critical role in the effect on appetite control. Ultimately, this means that the effect on the brain is mediated by afferent stimulation to the hindbrain (Blundell 2006).

There is also evidence that some of these potent chemical signals have a direct action on receptors in the brain. The arcuate nucleus (ARC), which contains two sets of neurons; one responsible for orexigenic, and the other anorexigenic activity, is the central site of action. Within the ARC are two interconnected groups of first order neurones that release neuropeptide Y (NPY) and agouti-related peptide (AgRP), in addition to anorexigenic substances pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). In close proximity to the NPY/AgRP neurons in the ARC are neurons producing peptide hormones called melanocortins. Substantial evidence indicates stimulation of NPY by various peripheral hormones leads to increased hunger or reduced satiety (leading to increased food intake), whereas increased satiety and reduced hunger (leading to decreased food intake) will occur following stimulation of the POMC/CART neurons via melanocortins (Chaudhri et al. 2006; Varela and Horvath 2012). These neurons reduce feeding activity by action at specific melanocortin receptors (melanocortin 3 and melanocortin 4 receptors) on downstream neurons. This second stage activity is facilitated by neuronal pathways comprising orexins and melanin-concentrating hormone in the lateral hypothalamic area. Consequently, at this level, the activation and inhibition of eating are embodied in distinct molecules with distinct neuronal connections. Both ghrelin and peptide PYY (3-36) are believed to exert at least part of their action on feeding by an interaction with NPY orexigenic neurons (Blundell 2006; Maier et al. 2008). **Figure 1** indicates how these episodic signals can influence the orexigenic drive embodied in neuropeptide Y (NPY) and AgRP neurons.

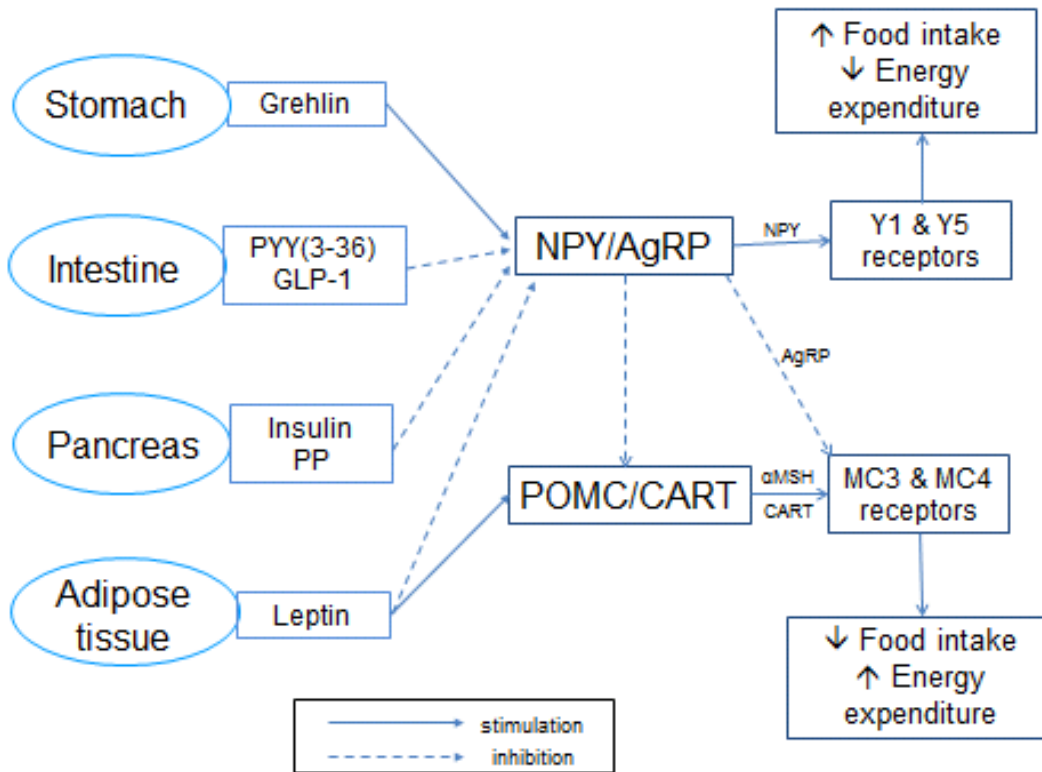


Figure 1. Circulating hormones influencing energy homeostasis via the arcuate nucleus. AgRP, agouti-related peptide; CART, cocaine-and-amphetamine-related transcript; GLP-1, glucagon-like peptide-1; α MSH, alpha-melanocyte-stimulating hormone; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; PYY, peptide YY. Diagram prepared using information from Benelam (2009).

Integration between the episodic and tonic signals is also achieved at this level because the NPY and melanocortin neurons are influenced by both leptin and insulin that signal the state of energy-related metabolic activity. The system is said to be leptin dependent. Leptin, providing a signal from adipose tissue, inhibits NPY neurons and stimulates the melanocortin system (Varela and Horvath 2012; Zhou and Rui 2013). In principle, therefore, high levels of leptin, reflecting large adipose stores, contribute to energy homeostasis by suppressing appetite. In practice, because obesity is characterized by the phenomenon of leptin resistance, this appetite-suppressing action may be quite weak.

2.2.2 Short-term regulation of energy intake

2.2.3 Appetite

There are two definitions of appetite in circulation; firstly, appetite covers the whole field of food intake, selection, motivation and preference, and secondly, refers specifically to qualitative aspects of eating, sensory aspects or responsiveness to environmental stimulation that can be in contrast to the homeostatic control of eating (Blundell et al. 2010). Appetite is commonly described as the desire to consume food and is experienced as perceived hunger, desire to eat, urge to eat, and/or prospective food intake. Therefore, appetite control is said to be the summation of perceived appetite and satiety sensations that ultimately lead to whether food is or is not consumed (Leidy and Campbell 2011).

The control of appetite can be conceptualized with reference to the satiety cascade, a theory originally developed by Blundell et al. (1987). This cascade provides a means of describing the form of eating patterns, highlighting the involvement of satiation and satiety. Food intake is regulated by cognitive, sensory, post-ingestive and post-absorptive processes, all of which are dictated by signals initiated from the onset of one eating episode and continuing to the next. The satiety cascade is illustrated in **Figure 2**, which highlights how psychological and physiological stimuli arising from the consumption of a food modulate the effects of that food on appetite sensations and the pattern of eating.

In this thesis, “*appetite*” is used as a general expression referring to the overall sensations associated with food intake, if not otherwise stated.

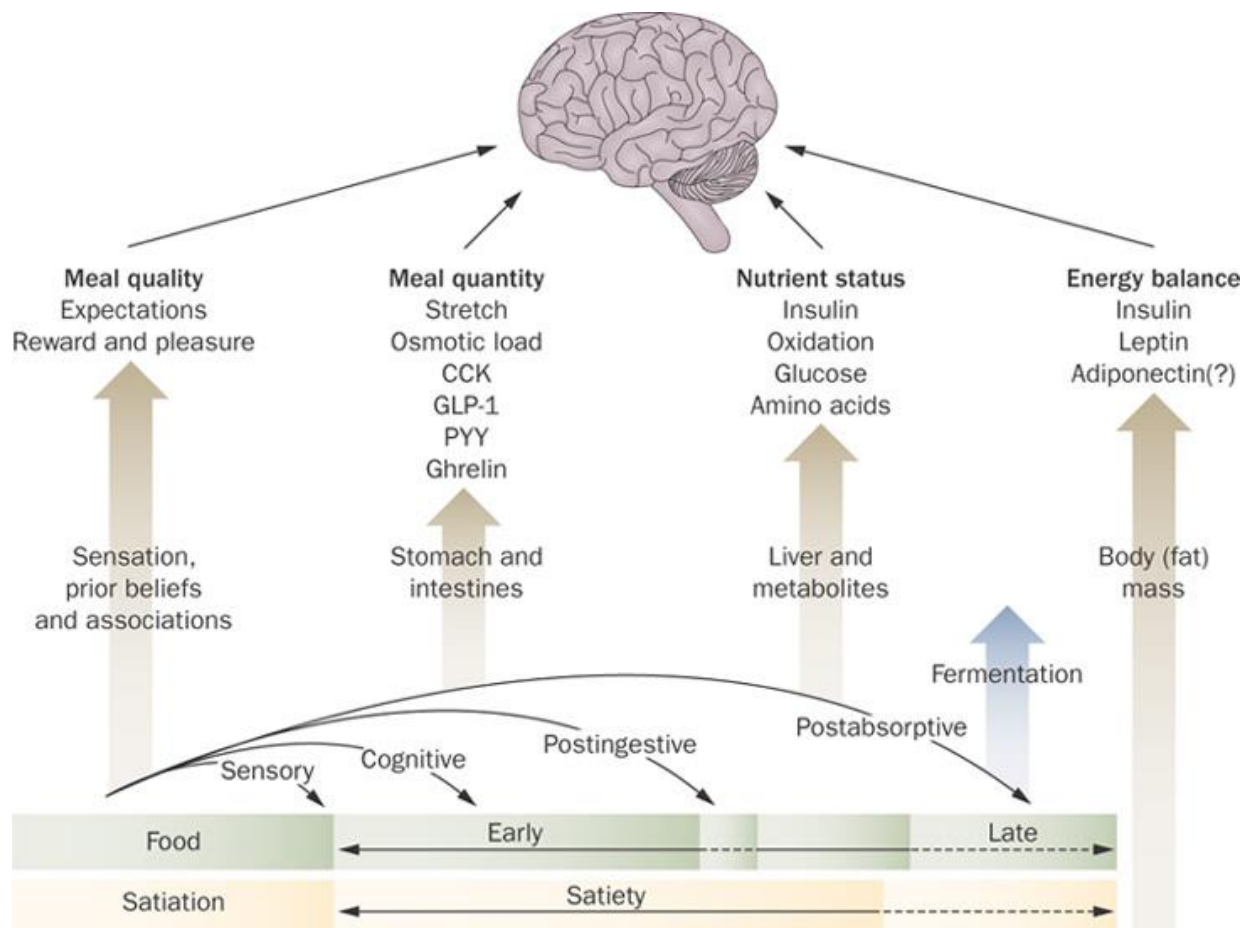


Figure 2. Satiety cascade showing the relationship between satiation and satiety, and some mediating psychological and physiological processes. Satiety cascade from Blundell modified by Mela (Mela 2006).

2.3 Appetite sensations

2.3.1 Hunger and desire to eat

“Hunger” is the concept or intervening variable that generates the conscious sensations for the drive to eat, and is accompanied by physical sensations in parts of the body, such as stomach or head. In its strong form hunger may include feelings of emptiness in the stomach or feelings of light headedness (Blundell et al. 2010).

Although hunger is not directly measurable it can be subjectively rated in magnitude. Hunger is associated with food deprivation, but it is not always determined by it, thus it can be seen as the phase between physiological state and food consumption. Subjective hunger represents an intermediary step in the cause-effect sequence between gut filling and cessation of meal ingestion (de Castro and Elmore 1988). Moreover, hunger plays an important role but is not the only factor in determining food intake (Mela 1999).

“Desire to eat” is problematic to determine, since this perception is regularly used as synonym for hunger in the current literature. Nonetheless, this feeling refers to the readiness to accept food primarily because of their rewarding and pleasurable characteristics (Kissileff and Van Itallie 1982). Desire to eat can be triggered even though an individual is highly satiated, because of its link with sensory-specific satiation (SSS) (Benelam 2009). It can therefore be considered as an important determinant of subsequent food consumption.

2.3.2 Satiety and fullness

The general concept of “satiety” consists of sensory, cognitive, post-ingestive and post-absorptive aspects. It can be divided into two distinct functions, “satiation” and “satiety”. Satiation or “intrameal satiety” refers to a process that promotes the termination of a meal (eating) thus controlling meal size. Satiety on the other hand, also referred to as “inter-meal satiety” or “post-ingestive satiety”, is the process that leads to inhibition of further eating indicated by reduced postprandial hunger and/or increased fullness after a meal has finished (Blundell et al. 2010). Synchronicity of neural and hormonal signals which originate from the mouth and upper GI tract in response to the physicochemical properties of ingested food relate to satiation, whereas satiety may result more from post-absorptive metabolic processes and their metabolites which operate during an inter-meal basis (Langhans and Geary 2010).

Satiation and satiety are closely connected to and influenced by the sensory aspects of food and learning processes through both their association with environmental cues and physiological, psychological and social consequences during and after eating (Blundell et al. 2010). Metabolic satiation and satiety refers to

all postprandial physiological mechanisms (e.g. hormonal and neural signals, and GI motility) via the gut-brain axis and its close relationship with energy homeostasis.

The term “fullness” can be defined as a sensation reflecting the degree of stomach filling (Sorensen et al. 2003).

2.3.3 Sensory specific satiation

Sensory specific satiation is a phenomenon that relates to the decline in pleasantness of a food as it is eaten relative to ‘uneaten’ foods that have different sensory qualities (Rolls et al. 1981; Wilkinson and Brunstrom 2016). This decline in pleasure or boredom with a food on repeated exposure occurs when there has been little opportunity for digestion and absorption, and is related primarily to the sensory aspects of the food. Texture, flavour, and colour influence the degree of sensory specific satiety. Sensory specific satiation plays an important role in food intake as it is thought to promote both the termination of an eating episode (Hetherington 1996) and the tendency to resume eating when different foods are made available (i.e., desserts) (Rolls et al. 1981). For example, in a study by Rolls et al. (1984), individuals consumed 60% more calories of a multiple-course meal compared to a single-course meal.

Thus, the body has a complex network of signals involved in the development of satiation and satiety. However, for free-living humans, choices about what and how much to eat are affected not only by internal appetite signals such as satiation and satiety but also by many other factors including the palatability of the food in question, the portion size provided, the time of day and the presence of other people (Bellisle 2003).

Because of the significant role chemical messengers have in the control of eating, it is necessary to discuss basic aspects related to chemical signalling. Many GI chemical signals involved in eating control have a classical endocrine mode of action. Specialized cells synthesize the signal molecule and in response to particular stimuli will secrete it into extracellular space. From here, the signal molecule diffuses into local capillaries, travels in the blood and finally binds to specific receptors that initiate its biological action. Some

GI chemical signals have a paracrine mode of action, which differs in that the signal molecule acts locally, reaching target cells before entering the blood (Langhans and Geary 2010). A few GI chemical signals have both paracrine and endocrine modes of action. It is important to highlight that circulating levels of GI chemical signals are often found in much higher concentrations in the hepatic portal vein (HPV) than in the general circulation, a factor which is important to consider when assessing physiological actions of GI signals that act locally in the liver. Additionally, another complexity originates from endocrine signals that act in the brain to influence eating. Brain levels of hormones and metabolites are not reflected in plasma levels because of the selective barrier and active transport features of the blood-brain-barrier (BBB).

Because endocrine signals appear in the systemic circulation, they have been the focus of intensive investigation. The myriad of research has often utilized sets of explicit empirical criteria modelled on endocrinological concepts to determine which endogenous endocrine signals are normally involved in eating control, playing both physiological and pharmacological roles. Pharmacological evaluation of signals is important as therapeutic agents can be based on either physiological or pharmacological actions of particular signals.

The following sections discuss GI signals that are currently considered key players in the physiological control of eating, including evidence supporting agonist/antagonist roles.

2.4 Gut peptides; secretory controls and physiological roles in eating

The total volume of food eaten and glycaemic control are vitally dependent on the control of and physiological responses to individual meals, and a significant part of these functions is suggested to be mediated via ghrelin, CCK, GLP-1 and PYY (3-36) secretion (Steinert et al. 2017). Timing, size and content of meals provide a full description of what, when and how much food is eaten. Ghrelin, CCK, GLP-1 and PYY (3-36) contribute to the three acknowledged motivational processes that provide the basic unconditioned control of meal initiation and size; hunger, satiation and postprandial satiety.

It is well recognized that meal-stimulated insulin release constitutes for approximately half of total daily insulin secretion (Kruszynska et al. 1987; Polonsky 1988), and more recently measurement of glycated haemoglobin (HbA1c) in well-controlled T2DM patients showed that meal-related increases in blood glucose account for around 70% of the total rise in day time blood glucose levels over fasting levels (Monnier et al. 2003). Two of the fundamental factors related to meal-induced increases in blood glucose are GE, which determines the rate of appearance of glucose in the small intestines and blood, and the release of incretin hormones, which are GI hormones that stimulate insulin secretion. Since CCK and PYY influence GE, and GLP-1 together with gastric inhibitory polypeptide (GIP) are the two main incretin hormones, collectively they all exert effects that mediate meal-related glycaemia, thus meal physiology is an essential factor of glycaemic control (Steinert et al. 2017).

Local signalling in the GI tract can occur in one of three ways. Firstly, hormones may behave in a paracrine mode, i.e., be released from the lamina propria and act on surrounding non-neural cells before absorption. Secondly, they may follow a neuroendocrine-like mode if they affect neural afferents in the lamina propria. Thirdly, hormones may act in a neuroendocrine-like fashion subsequent to release from axon-like cytoplasmic extensions of enteroendocrine cells, known as neuropods (Steinert et al. 2017). **Table 1** describes the normal, endogenous physiological functions of hormones in terms of meal-related roles. Criteria one and two address the likelihood that the candidate signal controls a particular function. Criteria three to five deal with the candidate signal's sufficiency, whereas criterion six concerns specificity. For hormones exerting an endocrine mode of action, endocrine tests of criteria one may be based around concentrations of the molecule in blood and its site of action. Criteria three, four and six may be tested IV infusions (unless the BBB inhibits access). Plasma levels and IV infusions do not provide adequate tests of paracrine or neuropod signalling because IV infusions of a hormone may not mimic its concentration at paracrine or neuropod sites of action, even if there are adequate hormone levels administered at concentrations similar to meal-related changes seen in blood. Therefore, for paracrine or neuropod modes of action, the criteria remain only theoretical, as there is currently no validated means to administer

hormones locally into the lamina propria or to neuropods, and no means of quantifying their concentrations at these sites.

Due to the harsh environment of the gut, oral delivery of peptide hormones has been unsuccessful on the whole as peptides cannot enter circulation via the intestinal wall. The subcutaneous route is therefore the established route for peptide hormone delivery; however, it should be considered that oral administration of gut peptides better mimics the physiological post-prandial state (Varamini and Toth 2016). Subcutaneous administration does not allow for consideration of local site of action (local vagal afferents) or absorption into the portal circulation before systemic actions occur (de Silva and Bloom 2012).

Table 1. Criteria to assess physiological status of GI hormones in meal-related functions. Taken from Steinert et al. (2017)

Criteria to assess physiological status of GI hormones in meal-related functions
1) Concentrations of the hormone change at the site of action in a pattern consistent with the effect.
2) Cognate receptors for the hormone are expressed at its site(s) of action.
3) Exogenous administration of the hormone in amounts duplicating the meal-related changes in endogenous patterns at the site of action produces an effect.
4) Administration of secretagogues for the hormone produce effects similar to the effect of the hormone.
5) The hormone's effect occurs in the absence of abnormal, behavioural, physiological or subjective effects.
6) Administration of selective agonists and antagonists of the hormone's receptors produce effects that are consistent with their receptor pharmacologies.

Multiple parameters of hormone secretion other than plasma concentrations then may determine feedback signals that control eating. These parameters include times of onset of changes in plasma levels, rates of change and effects of sustained or integrated levels versus momentary levels (Steinert et al. 2017). However, the roles of acute endogenous physiological signals have not been widely investigated. Instead, the focus of research has been on one single parameter, the peak plasma level, and peaks have been modelled only loosely by continuous infusions that do not consider the duration or timing of peaks. With reference to criterion one, endocrine doses are tentatively defined as those reproducing the peak plasma levels produced by mixed-nutrient meals. Identifying hormone function with the aid of agonists or

antagonists (criterion 6) is associated with advances in receptor pharmacology and receptor sub-type analyses. Using antagonists is now considered a core element in identifying physiological responses. This method requires careful interpretation if the biological half-life, receptor affinity, or access beyond the BBB differ between the target hormone and agonist/antagonist. **Table 2** summarizes the evidence relating to peak physiological doses of the key peptide hormones following mixed nutrient meals.

Table 2. Physiological endocrine infusions of ghrelin, CCK, GLP-1 and PYY (3–36) in healthy-weight humans

Hormone	Physiological dose (pmol/kg/min)	Reference
Ghrelin	? (<0.3)	Lin <i>et al.</i> 2004; Theodorakis <i>et al.</i> 2006
CCK	0.2-0.7	Liddle <i>et al.</i> 1985; Ballinger <i>et al.</i> 1995
GLP-1	0.3-0.9	Gutzwiller <i>et al.</i> 2004; Gunnarsson <i>et al.</i> 2006
PYY (3-36)	? (<0.2)	Deane <i>et al.</i> 2010; Laurenus <i>et al.</i> 2012

2.4.1 Ghrelin and Eating

Produced predominantly in the stomach, ghrelin has been identified as the only known orexigenic gut hormone identified to date, referred to as the ‘hunger hormone’ (Patterson *et al.* 2011). Ghrelin O-acyltransferase is required to catalyze the conversion of ghrelin into its biologically active acylated forms, octanoyl- and decanoyl-ghrelin, together referred to as acyl-ghrelin. Less than 10% of circulating ghrelin

is acyl-ghrelin (Kirchner et al. 2012), assaying of which is problematic (Prudom et al. 2010), and therefore complicates investigations using endogenous ghrelin. Plasma ghrelin levels are reported to increase progressively prior to the onset of meals and fall quickly afterwards (Spiegel et al. 2011; Gibbons et al. 2013). Fluctuations in plasma ghrelin around lunch and spontaneous dinner were closely related to hunger ratings in a group of healthy-weight, time-blinded males, as described by Cummings et al. (2004). This was also shown in study by Gibbons et al. (2013), who report that ghrelin was significantly associated with changes in hunger and food intake in overweight and obese adults. Blom and colleagues (2009) report that lunch request was preceded by an increase in ghrelin, reaching at least 93% of fasting values, following a standardized breakfast in men. They also found that the area under the curve (AUC) decrease in ghrelin response under the time-blinded conditions correlated with breakfast-lunch inter-meal intervals, suggesting that ghrelin responses are related to meal initiation. Ghrelin concentrations at meal onset have also been reported to correlated with meal size in both normal and overweight individuals (Gibbons et al. 2013). These findings provide evidence for ghrelin to fulfil criterion 1 of **Table 1** for ghrelin levels around meals to play a role in hunger signalling. Investigations involving the use of ghrelin infusions have so far, however, been unsuccessful in meeting criterion 3 in **Table 1**.

One hour after a standardized meal there was no effect of a near physiological dose of ghrelin reported in normal weight males who received either 0.3 pmol/kg/min ghrelin or saline infusions on hunger ratings or food intake assessed by *ad libitum* eating (Lippl et al. 2012). There was also no effect on meal size, or timing of the spontaneous meal interval, suggesting that 0.3 pmol/kg/min was not potent enough to influence the regulation of food intake. Supraphysiological doses however have been shown to stimulate eating. Exogenous infusion of ghrelin at a dose of 5.0 pmol/kg/min increased food intake at a buffet meal by 28% in normal weight human subjects (Wren et al. 2001). In order to highlight any difference in sensitivity to ghrelin between the lean and obese groups, Druce and colleagues (2005) tested both lean and obese individuals who received infusions of either ghrelin 5.0 pmol/kg/min (high dose), ghrelin 1.0 pmol/kg/min (low dose) and saline alone. The low-dose infusion of ghrelin increased *ad libitum* energy

intake at a buffet meal in the obese group only (increase of 166 ± 46 kcal), whereas high-dose ghrelin infusion increased energy intake in both groups. In the obese group, the mean increase was 381 ± 100 kcal, whereas in the lean group the mean increase was 118 ± 47 kcal, compared to saline. These findings indicate that ghrelin sensitivity is retained in obese individuals and therefore ghrelin receptor antagonists may prove to be useful appetite reducing agents. Further studies of the effects of ghrelin antagonists in both normal weight and obese humans are warranted, however there is some evidence of reduced food intake in rodents following administration of ghrelin antagonists (Asakawa et al. 2003) but the effects of these agents in human subjects have not been investigated thoroughly as an anti-obesity agent. The role of ghrelin in appetite control can also be seen in subjects who have been administered antipsychotic drugs. Increases in circulating ghrelin are negatively correlated with weight in subjects receiving olanzapine (Hosojima et al. 2006; Kim et al. 2008) and clozapine (Lu et al. 2015), suggesting that benzodiazepine antagonists may modulate appetite via NPY/AgRP neurons.

With regards to food hedonics and nonhomeostatic control of eating, ghrelin may be influential to the eating process by increasing neuronal activity in brain regions associated with food reward. Data from Goldstone et al. (2014) showed that non-obese adults who received subcutaneous injections of acyl ghrelin before functional magnetic resonance imaging (fMRI) food-picture evaluation tasks had increased hippocampal activation to high-energy and low-energy-food pictures. Similar effects of ghrelin administration are also documented by Malik et al. (2008), who report increased neural responses to food pictures in the amygdala, orbitofrontal cortex, anterior insula, and striatum of nonobese individuals, all of which are areas implicated in encoding the incentive value of food cues. This evidence is particularly promising, and thus ghrelin may exert effects beyond homeostatic mechanism in controlling food intake.

2.4.2 PYY and Eating

The most important stimulus for PYY release in rodents is protein ingestion (Batterham et al. 2006) which is potentially similar in healthy humans, with high protein meals increasing circulating concentrations of PYY more than meals rich in carbohydrate or fat (Van der Klaauw et al. 2013). Yet the relative effect of

protein remains somewhat unclear, according to more recent findings (Heden et al. 2013; Van Avesaat et al. 2015). In obese individuals it has been reported by Essah et al. (2007) that a low-carbohydrate, high-fat diet induced the highest levels of PYY. Nevertheless, evidence indicates that PYY levels start to rise within about 15-30 minutes of any caloric ingestion (Gibbons et al. 2013) before the nutrients reach distal parts of the gut, possibly suggesting some other neural or hormonal mechanisms for its release (Marić et al. 2014).

There are two circulating forms of PYY, PYY(1-36) and PYY(3-36), the latter of which is most predominant and actively binds to Y2 receptors widespread throughout the brain, i.e., nucleus accumbens, amygdala, hypothalamus and hippocampus (Stanić et al. 2006). Rodent studies indicate that peripheral administration of a Y2 receptor PYY(3-36) agonist in rodents induces neuronal activation in multiple brain regions (Stadlbauer et al. 2013). However, difficulties in assaying PYY(3-36) confounds investigatory studies of its physiology. Since NPY and PYY are highly homologous (70%), residues 32–36 are identical and thus PYY may be subject to C-terminal degradation (Toräng et al. 2015). Additionally, postprandial PYY and GLP-1 profiles are often dissimilar because dipeptidyl peptidase-4 (DPP-4) activates PYY but has the opposite effect on GLP-1 (Steinert et al. 2017).

In humans, infusion of 0.8 pmol/kg/min of PYY(3-36) significantly decreased subjective hunger and reduced food intake by 33% over a 24 hour period in a study by Batterham et al. (2002). In this study, peak PYY(3-36) levels increased from ~8 to 44 pmol/L following 90 minutes of infusion, which reduced *ad libitum* eating when food was presented 2 hours after infusion ended. A previous study that monitored the effects of PYY levels following three different caloric loads, 530, 870 and 4500 kcal, showed an increase in PYY from ~9 pmol/L to ~12, ~25 and 55 pmol/L, respectively (Adrien et al. 1985). Thus, Batterham and colleagues (2002) concluded that administration of a supraphysiological dose of PYY(3-36) in their study elicited a physiological effect.

Similarly, Le Roux et al. (2006) report that a 0.7 pmol/kg/min PYY(3-36) infusion decreased meal size, but lower infusions of 0.5 and 0.6 pmol/kg/min did not. Instead the lower doses increased subjective fullness ratings. In the same study, a high calorie meal of 3000 kcal was given which increased PYY to ~40 pmol/L,

which was lower than the ~40-60 pmol/L increase following lower dose PYY infusions. It has been shown that lower doses of 0.2-0.3 pmol/kg/min had no eating-inhibitory effect, with no significant reductions in meals served subsequently to infusions (Sloth et al. 2007; De Silva et al. 2011). Therefore, the doses of PYY infused that did inhibit eating in the previous studies increased levels of PYY more than meals containing excessive calorie contents, suggesting that PYY(3-36) action does not support the criteria necessary for a physiological endocrine effect (Criterion 3 **Table 1**). This is further corroborated by Gerspach et al. (2011) who report no associations in PYY AUC after low-carbohydrate high-fat or high-carbohydrate low-fat breakfasts and subsequent energy intakes, yet GLP-1 and ghrelin were correlated with lunch intakes. Although not all studies report negative side effects from PYY infusions following standard meals, even at doses of 0.8 pmol/kg/min (Le Roux et al. 2006), adverse effects have been seen in subjects who have received PYY infusions whilst fasted, most commonly presenting symptoms such as nausea and malaise (Sloth et al. 2007; Torang et al. 2016). This suggests that more evidence is required to support criterion 5 in **Table 1** (i.e., the hormone's effect occurs without abnormal behavioural or physiological effects) as it appears the effectiveness of PYY on satiety is selective and warrants further investigation. To the researcher's knowledge, there is only one study that has explored the satiating effect of intrameal PYY(3-36) in humans. Degen and colleagues (2005) acknowledge that in order to reduce meal size a dose of 0.2-0.4 pmol/kg/min PYY(3-36) was required, and that both 0.2 and 0.4 doses increased PYY from fasting levels of ~10 to ~25 and ~31 pmol/L, respectively. The infusions increased PYY more than a 1500 kcal meal did, suggesting that the inhibitory effects of PYY administered during the inter-meal interval was pharmacological, not physiological. In addition subjects also reported to be nauseous following the 0.4 pmol/kg/min infusion. In the same study, an 8 pmol/kg/min infusion induced nausea in 50% of subjects, suggesting that reductions in meal size were most likely due to adverse effects, not the slight supraphysiological doses of PYY administered, thus these findings are not valid to meet criterion three or five in **Table 1**.

Studies of PYY-receptor antagonism are necessary to identify whether PYY is a plausible candidate for physiological satiation or a postprandial satiety signal, however currently methodological drawbacks, i.e., the absence of a specific PYY antagonists suitable for humans, make this a challenging task.

2.4.3 CCK and Eating

Cholecystokinin is the most established GI endocrine satiation signal in humans. This peptide is released after a meal from the enteroendocrine cells in the duodenum and upper small intestine (Steinert et al. 2017). Per calorie, oral lipids stimulate CCK most, followed by protein intermediately and carbohydrates least, according to studies using isoenergetic loads (Little et al. 2005; Ryan et al. 2013). The circulatory half-life of CCK is several minutes, yet CCK levels are increased 10-15 minutes after meals and reduced during fasting (Steinert et al. 2017). Cholecystokinin regulates GE, gall bladder contraction and pancreatic enzyme release (Schwizer et al. 1997; Dufresne et al. 2006), thus influencing the transit of a meal through the GI tract via the ‘ileal brake’ mechanism to optimize nutrient digestion and absorption. Studies have shown that CCK is one of the factors responsible for meal termination (Lieverse et al. 1994) and there is convincing evidence for its effects on reducing appetite and food intake.

When compared to saline infusions, there was a decrease in eating of around 317 kcal following infusions of ~0.6 pmol/kg/min of cholecystokinin-8 (CCK-8) in healthy individuals. Plasma concentrations increased from ~0.45 pmol/L to ~7.28 pmol/L immediately before the meal, which is a response that mimicked physiological post-prandial concentrations of CCK (Ballinger et al. 1995). Similar eating-inhibitory effects were also shown by Gutzwiller et al. (2004), with a reduction of almost 200 kcals following 0.2 pmol/kg/min infusions of CCK-33 in healthy men. Unlike PYY, this evidence suggests CCK fulfils criteria 3 and 5 of **Table 1**.

If a preload or meal has the potential to stimulate endogenous CCK release, this might result in supraphysiological plasma CCK concentrations from a combination of CCK infused and naturally released in exploratory studies. Therefore, to identify the effect of a determined dose of CCK on satiation, Lieverse

and colleagues (1995) used bananas as a preload food before administering CCK infusions, as ingestion of banana has been shown to have a minimal CCK stimulating potency (Lieverse et al. 1993). CCK secretion was not stimulated by the banana preload, and therefore the resulting reduction in food intake reported from *ad libitum* eating can be attributed to the infused CCK, synergism with endogenous CCK did not influence the eating-inhibitory effect. In addition to this, CCK receptor antagonism has also been shown to reduce subjective fullness ratings during meals, increase meal size and block the satiating effects of intraduodenal lipid delivery in several studies (Lieverse et al. 1994; Matzinger et al. 1999; Beglinger et al. 2001) providing firm evidence that administration of secretagogues and antagonists of CCK have physiological effects on meal-related functions (**Table 1**), exhibiting itself as a prime target in the endocrinological control of eating.

2.5 Glucagon-like peptide-1

2.5.1 Secretion

Glucagon-like peptide-1 is released in response to meals and enhances glucose-induced insulin secretion from pancreatic islets, hence its recognition as an incretin hormone (Yabe et al. 2018).

Glucagon-like peptide-1 is secreted by open-type enteroendocrine cells, originally identified as L-cells, located in both the small and large intestine (Polak 1971; Buffa et al. 1978). GLP-1 cells located in the distal jejunum and ileum coexpress and secrete not only GLP-1 but PYY, with some cells also coexpressing CCK, GIP and secretin (Egerod et al. 2012). Glucagon-like peptide-1 also acts as a neurotransmitter, produced by preproglucagon-expressing neurons in the NTS of the brain stem (Larsen et al. 1997). Since GLP-1 is secreted and released from two separate sites, it is important to distinguish the central action of gut-derived GLP-1 from that of brain-derived GLP-1.

Generally, the peripheral factors send their information to the brain through two distinct pathways, the humoral and neural pathways (**Figure 3**). The humoral pathway is composed of the BBB and the circumventricular organ (CVO) that has a 'leaky' BBB (Katsurada and Yada 2016). Kastin and colleagues (2002) report that GLP-1 can enter the brain via the BBB and radiolabeled GLP-1 binds to its receptors

expressed in the CVO. On the other hand, endogenous GLP-1 derived from the gut is rapidly cleaved by a proline/alanine-specific DPP-4, with its half-life being less than two minutes (Kieffer et al. 1995). Hence, it is probable that gut-derived GLP-1 influences the brain mainly through the neural pathway, which is composed of the vagal afferent fibres at the intestinal or hepatic portal area.

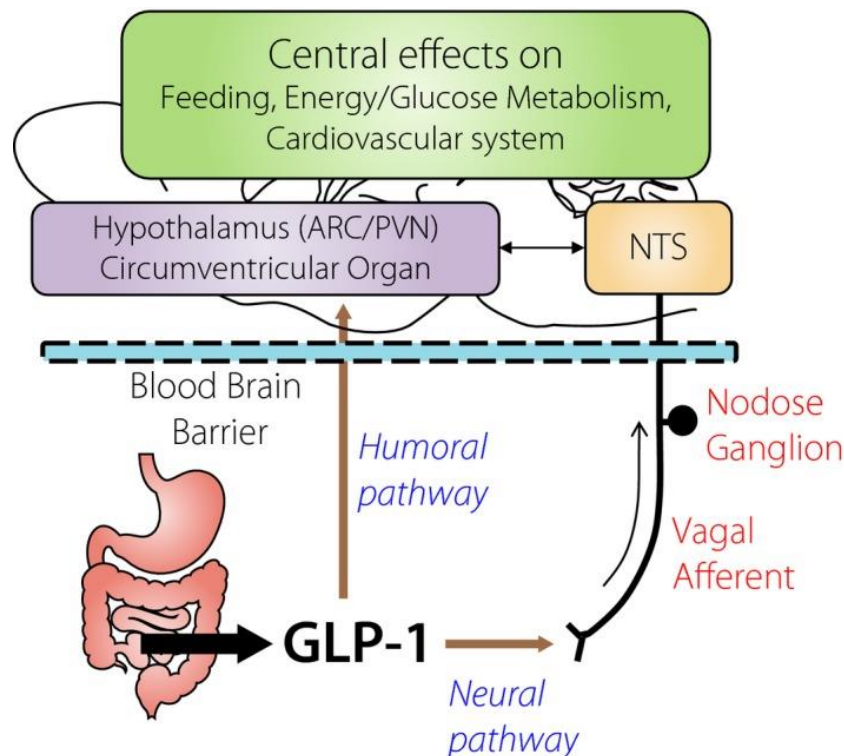


Figure 3. Glucagon-like peptide-1 (GLP-1), released from the intestine, informs the brain by passing through blood–brain barrier (humoral pathway) and by interacting with vagal afferent nerves (neural pathway). Consequent activation of the hypothalamus, circumventricular organ, and brain stem including nucleus tractus solitarius (NTS), regulates feeding, energy/glucose metabolism and the cardiovascular system. ARC, arcuate nucleus; PVN, paraventricular nucleus. Taken from Katsurada and Yada (2016).

In humans, two equipotent molecular forms of GLP-1 circulate, GLP-1(7-37) and GLP-1(7-36NH₂), the latter of which predominates. Dipeptidyl peptidase-4, found on the luminal surface of capillary endothelial cells, in the liver and in the blood, rapidly degrades active GLP-1 into inactive forms, GLP-1(9-37) and GLP-1(9-36NH₂). Current GLP-1 assay methods usually produce similar relative changes, but often differ

in absolute concentrations (Bak et al. 2014; Kuhre et al. 2015). Glucagon-like peptide-1 secretions are best estimated by the sum of active and inactive forms in plasma because of the rapid degradation of active GLP-1 by DPP-4 (Holst 2007; Moller et al. 2011). Plasma concentrations of GLP-1 are at their lowest during an overnight fast and increase rapidly during meals. Usually, GLP-1 does not return to basal levels between meals. For example, following a standardized breakfast, a group of healthy males and females consumed mixed-nutrient lunches of 511, 743 and 1023 kcal, to which active GLP-1 increased from 5 pmol/L to ~9, ~12 and ~16 pmol/L, respectively, at 30 minutes and did not decrease below ~7 pmol/L after 3 hours (Alsalim et al. 2015).

Glucose and several other carbohydrates usually bring about monophasic increases in GLP-1, initial rises after 5-15 minutes, peaking at 15-30 minutes and returning to initial values after 3-4 hours (Steinert et al. 2017). Following mixed-nutrient meals, GLP-1 levels can often follow biphasic patterns, with secondary peaks 60-120 minutes. Gastric emptying rates (GER) and digestibility of meal contents most likely give rise to this, along with the impact of individual nutrients. With regards to macronutrients, protein and lipids normally produce a slower-onset and sustained increase in GLP-1 when compared to glucose. Due to both mono and biphasic patterns in GLP-1 secretion, it is difficult to identify whether any macronutrient should be deemed the most potent secretagogue. Additionally, both basal and glucose-stimulated GLP-1 secretion are released in an episodic manner, with a period of ~8 minutes, according to Balks et al. (1997).

From a mechanistic perspective, there are several factors which may promote prompt release of GLP-1. First, the location of GLP-1 cells in the duodenum and proximal jejunum allows direct stimulation of GLP-1 as soon as the ingesta exits the pylorus. Second, the rate of GE of glucose, which is more pronounced in fasted individuals, may produce glucose concentrations that exceed the absorbency capacity of the proximal small intestine, which means glucose may reach the distal GLP-1 cells within 5-10 minutes following meals. Third, the rate of increase in plasma GLP-1 may exceed the rate of glucose influx, suggesting that

neuroendocrine reflexes may stimulate GLP-1 secretion in addition to direct stimulation of GLP-1 cells by glucose.

Intraduodenal-infusion studies report that carbohydrate increases plasma GLP-1 quicker than either protein or lipids (Beglinger et al. 2010), and that biphasic responses sometimes occur (Little et al. 2005; Pilichiewicz et al. 2007). Moreover, increasing the caloric loads of protein (Ryan et al. 2013), fat (Little et al. 2005) and carbohydrates (Pilichiewicz et al. 2007) increases GLP-1 levels. With regards to glucose infusion rates, GLP-1 responses were larger under conditions where the infusion rates were initially faster and then subsequently slower compared to identical loads infused at constant rates. For example, Chaikomin et al. (2005) investigated whether two conditions, varying in infusion rates, would influence incretin hormone release. The first condition involved infusion of glucose at 6 kcal/min for ten minutes, followed by 0.55 kcal/min for the remainder of the 2-hour infusion period, which was compared to condition two, a constant infusion of 1 kcal/min over two hours. Despite greater incretin responses, including GLP-1 over the first 75 minutes, there were no differences in circulating glucose levels between the two conditions. Increasing the initial intraluminal nutrient load increased GLP-1 secretion, but had no effect on the glycaemic response. Because GER are initially faster, the effects reported in this study may be as a result of the influence of GE and GLP-1 secretion. It would have been interesting to identify if the increase in circulating GLP-1 would have led to a decrease in *ad libitum* eating following the 2-hour infusion period, however this was not the focus of this study by Chaikomin and colleagues.

A vast amount of evidence highlights the importance of the distal small intestine in sustained GLP-1 secretion, as demonstrated by comparisons of GLP-1 secretion and glucose absorption. The threshold intraduodenal glucose infusion rate for sustained increases in GLP-1 was reported to range between 1-2 kcal/min (Schirra et al. 1996; Trahair et al. 2012) and 2-4 kcal/min (Pilichiewicz et al. 2007). The absorptive capacity of the duodenum and first 25-30 cm of the jejunum is estimated to be approximately 0.9-1.4 kcal/min (Modigliani et al. 1971; Pfeiffer et al. 1993). It can be proposed that glucose probably reached the distal jejunum in these studies when at least 1.5 kcal/min of glucose was delivered (Steinert et al. 2017). This suggests that stimulation of distal GLP-1 cells is necessary to prompt sustained GLP-1 secretion.

Modifying the length of the small intestine exposed to glucose on plasma GLP-1 was investigated by Little et al. (2006). On one occasion, glucose was infused at 3.5 kcal/min into the duodenum of healthy males over a 1-hour period into an isolated 60 cm segment of the proximal small intestine (“short-segment infusion”) and on a second occasion, the same amount of glucose was infused with access to the entire small intestine (“long-segment infusion”). The authors report larger rises in blood glucose and plasma insulin in addition to increases in plasma GLP-1 during the long-segment infusion only, suggesting that the release of GLP-1 is dependent upon more than 60 cm of the intestine being exposed to glucose. In a more recent study by Wu et al. (2015), 2 kcal/min of glucose was infused either via intraduodenal catheters that ended 12 cm distal to the pylorus or via intrajejunal catheters that ended 50 cm distal to the pylorus. The study reports that GLP-1 levels significantly increased more when glucose was delivered intrajejunally. The studies by Little et al. (2006) and Wu et al. (2015) suggest that there are optimal sites located within the small intestine that, if stimulated, can exert a sustained GLP-1 response. This is further corroborated by several other studies who report increases in GLP-1 secretion when carbohydrates were administered alongside acarbose, which delays the breakdown of sugars and starches and thus increased the amount of starch and sugars reaching the distal area of the small intestine (Seifarth et al. 1998; Gentilcore et al. 2005).

Secretion of circulating endogenous total and/or active GLP-1 in rodents have mainly been observed in response to intragastric or intrainestinal infusions of liquid diets or nutrient solutions and the results of which are generally similar to the findings in humans (D’Alessio et al. 2007; Yoder et al. 2009). Sampling from the hepatic portal vein (HPV) allows measurement of GLP-1 close to the site of its release. This is virtually impossible in humans however can be done in rodent models. As a result of first pass degradation in the liver, HPV measurements in rodents better reflect GLP-1 release in comparison to systemic measurements sampled in humans. Punjabi and colleagues (2014) compared parallel measurements of active GLP-1 from both the vena cava and HPV of rats subsequent to eating a regular chow meal. Acute increases of active GLP-1 were reported only in plasma taken from the HPV sampling location. Given the high dietary fibre, low fat and readily digestible carbohydrate content of the chow meal, these results may

not be surprising as the meal itself is not a strong stimulus for GLP-1 release. However, the study does suggest that following the chow meal, there may have been local paracrine effects in the HPV but no effects systemically.

In an investigation by Arnold et al. (2012), intestinal lymph sampling in rats following isocaloric high-fat or high-carbohydrate meals showed an increase in intestinal lymphatic concentrations of GLP-1. There was a marked prandial increase in GLP-1 following the high-fat meal when compared to the high carbohydrate meal, which is consistent with evidence supporting the stimulatory effect of fat on GLP-1 release and supports the hypothesis of a local paracrine effect of endogenous GLP-1 on intestinal afferents. Transient but supraphysiological increases in GLP-1 concentrations sampled from the vena cava of rats were reported following HPV infusions of GLP-1 at a rate of 1 nmol/kg/min. There were also reductions in meal size by almost 40% following GLP-1 infusions via the HPV (Punjabi et al. 2014). The findings indicate that the satiation response to such infusions may reflect a pharmacological rather than a physiological effect. Despite this, the effect may still potentially be relevant from a pharmacological perspective, such as GLP-1 receptor agonists.

GLP-1 receptor agonists and antagonists, will be discussed throughout the following sections in order to highlight the physiological roles of GLP-1. Exendin-4, exendin 9-39 and exendin 3-39, the latter of which is a truncated version of GLP-1 agonist exendin 4, will be discussed in context of GE, eating and glycaemic control.

2.5.2 Gastric mechanoreception

The stomach is richly innervated with mechanoreceptors that respond during and after meals, signalling the brain via vagal and splanchnic visceral afferents. In animal models, the effects of gastric mechanoreceptor signalling on eating has been explored. Gastric cannulas, which allow fluids to be infused or drained from the stomach, along with pyloric cuffs, which can be inflated to prevent food from entering the small intestine, have been used in rat models. Evidence indicates that when gastric cannulas are used to prevent ingested liquid food from accumulating in the stomach, meal size is increased considerably, and when

ingested food is prevented from entering the intestines, meal size is about normal (Philips and Powley 1996; Eisen et al. 2001). When fluid loads are infused into the stomach of rats with closed pyloric cuffs, eating is inhibited in proportion to the volume infused (Kaplan and Moran 2004). The effect of gastric fill on eating is identical with nutrient and non-nutrient loads. The evidence from rat model studies indicates that gastric volume is an adequate stimulus for mechanoreceptors to contribute to the control of eating (Eisen et al. 2001). Although, the pyloric cuff model does not fully assess the contribution of gastric mechanoreceptor involvement in the control of eating. The prevention of normal intrameal GE under the cuff close condition produces abnormal increases in gastric volume after termination of the meal. The closed cuff condition also stops any interaction between gastric and postgastric signals (Langhans and Geary 2010). Data highlights that such interactions are normally important, thus gastric signals may not be solely responsible to control meal size but they may however be important contributors (Chambers et al. 2013).

Inflation of a gastric balloon before meals has been shown to increase feelings of fullness and reduced meal size in normal weight and obese subjects. Sonographically measured antral cross-sectional areas after meals, but not fundal areas, were correlated with end of meal fullness and size of the next meal in a study by Sturm et al. (2004). This finding suggests that the crucial signal may be related to antral volume rather than fundal volume. However, to what extent pure mechanical distension of the gastric fundus and antrum can alter food intake remains unknown. In a study by Oesch et al. (2006), neither gastric fundus nor antrum distension showed a reduction in calorie intake in healthy males. Mechanical distension prior to eating had only a limited effect on hunger and fullness ratings, which may explain why total calorie intake after gastric distension was not reduced compared to the control treatment. Thus, it can be suggested that mechanical distension of the stomach only plays a role in triggering satiety as long as mechanoreceptors are stimulated.

Understanding the physiological functions of the GI tract involved in the digestion process, where food undergoes major transformation from undigested food to absorbable nutrients via mechanical and chemical breakdown, is important in identifying the postprandial metabolic and hormonal effects of food and its components.

Following ingestion and chewing, peristaltic waves deliver the food bolus to the stomach, which is the site after the oral cavity to strongly stimulate physiological signals related to food intake. Not only does the stomach store the undigested food but also vigorously processes it, participating considerably in the breakdown of food before finally emptying the processed fluid content with relatively homogenous properties into the duodenum for further breakdown by digestive enzymes (Langhans and Geary 2010). A critical step in regulating postprandial digestion and absorption to achieve long-term metabolic stability and control is GE. Gastric functions initially reduce the size of solid bolus particles and fat globules, adjusting the pH, caloric density, osmolality and viscosity of liquids (Leiper 2015). Gastric emptying is influenced by several interconnected and complex factors including neural regulatory mechanisms, hormonal influences and physiochemical properties of the food bolus.

The physical state of food, such as whether or not the food is solid or liquid, distinctively affects the integrated function of different gastric compartments, resulting in a distinctive GE pattern. Generally, liquids empty faster from the stomach whilst the emptying of solids is lengthier. Inherent characteristics of food, such as energy density, meal size, viscosity, pH and exercise are all factors that affect GER. Evidence from Kristek et al. (2010) and Marciani et al. (2001) highlight that GER are decreased more after ingestion of digestion-resistant foods structures and after more viscous, energy dense meals. Because of the complexity of the mechanisms that control the GE process, it is not surprising that there is considerable interindividual variation in emptying rates (Maughan and Leiper 1996; Paintaud et al. 1998). However, most individuals do appear to be relatively consistent in the rate at which they empty the same solution on different occasions (Leiper 2015).

Body posture is also important because it modulates physical intragastric conditions and the dynamic emptying process. For example, in a seated position hydrostatic pressure, modulated by gastric tone, dictates GE, of which gravity influences intragastric distribution and emptying rate (Horowitz et al. 1993; Steingoetter et al 2006). Gastric emptying rate is ultimately affected by the physiochemical properties of meals that affect the chemical environment of the gastric lumen. The nervous and enteroendocrine systems

have a vital impact on the control of GE and intestinal motility, which then subsequently affect appetite sensations and energy intake (Janssen et al. 2011). Gastric and postgastric mechanisms are involved, such as gastric distension activates GLP-1 neurons located in the nucleus of the solitary tract, suggesting that GLP-1 is involved in the gastric distension-induced regulation of appetite.

Inhibition of food intake by CCK is enhanced following stomach distension. Several other GI-derived peptides, CCK, GLP-1, PYY and ghrelin, also influence GE, where the former reduce the rate and ghrelin exerts the opposite effect. Postgastric feedback mechanisms, such as the so-called 'ileal brake' mechanism induced by PYY, alter the gastric outflow rate to meet digestion and absorption capacity of the upper sections of the small intestine. In addition, appetite sensations are also affected by gastric motility. The main determinant underlying the gastric satiation and satiety is based on mechanosensitivity in which gastric distension and accommodation are the key factors. Sepple and Reid (1989) also suggest that a reduction in gastric distension may play a role in the initiation of postprandial hunger. Although the intestines, like the stomach, are sensitive to distension, most research agrees that the sensitivity of intestinal contents is mainly based on mucosal recognition of luminal content (Janssen et al. 2011). The detection of the energy and nutrient content of a meal by the stomach most likely plays only a small role in controlling appetite. Yet despite this, Steinert and colleagues (2012) suggest that if the gastric phase of eating is bypassed this has negative effects on acute appetite control in that hunger was reported to be less suppressed and fullness lessened following delivery of nutrients directly to the duodenum of healthy subjects. Therefore, gastric motility is an important determinant for hunger, satiety and satiation. Mechanisms related to different sensations of appetite may depend on the site of the gut exposed to nutrients. Gastric satiation appears to be more volumetric and intestinal satiation nutritive. Both gastric motility and nutrient exposure are necessary for the control of appetite sensations, however the role of intestinal exposure to nutrients is emphasized as the stomach empties (Janssen et al. 2011).

2.5.3 Gastric emptying and GLP-1

There is evidence to support the role of GLP-1 in GE endocrine control. Physiological doses of GLP-1 (0.4 pmol/kg/min) slowed the GER of liquids in healthy individuals in a study by Nauck et al. (1997). This is also supported by Wettergren and colleagues (1993), who reported a 50% decrease in GER (from 16 ± 2 min to 30 ± 5 min) in healthy volunteers, supporting criterion three in **Table 1**. There is also evidence to support criterion six with regards to GLP-1 receptor antagonism. Deane et al. (2010) report accelerated GE following administration of exendin 9-39, a GLP-1 receptor antagonist, in healthy males and females. Following a solid mashed potato meal, subjects who received exendin 9-39 showed significantly faster stomach emptying than the placebo, 68 ± 8 min and 83 ± 7 min, respectively. More recently, Aulinger et al. (2014) also reported similar effects of accelerated GE following exendin-9-39 antagonism in subjects with T2DM. With regards to the literature, failure of GLP-1 receptor antagonism to affect GE has been identified in three studies (Salehi et al. 2008; Nicolaus et al. 2011) even after supraphysiological doses of exendin 9-39 were administered (Nagell et al. 2007). It is important however to acknowledge that these three studies did differ in factors that may lead to variable GE outcomes, such as test meal characteristics. For example, in the study by Nagell et al. (2007) a liquid meal was consumed, whereas Nicolaus and colleagues (2011) provided a semisolid meal. Differences in stomach emptying rates following liquid and solid meals are well established. Exendin 9-39 has also been reported to stimulate PYY (3-36) secretion (Wu et al. 2013; Steinert et al. 2014), which may have contributed to the slowing of GE, thus given rise to conflicting findings in the aforementioned studies. There is also research in favour of GLP-1's role in intestinal motility. IV infusion of physiological doses of GLP-1 has been reported to stimulate tonic and phasic pyloric pressures and reduced antral and duodenal pressure waves in a study by Schirra et al. (2000). In addition, glucose-induced changes in antropyloroduodenal pressures were diminished following exendin 3-39 administration (Schirra et al. 2006).

2.5.4 GLP-1 and its role in eating

There is evidence for GLP-1 to fulfil criterion 3 in **Table 1** as an endocrine satiation signal in humans. Intravenous infusions of physiological doses of GLP-1 has been reported to reduce energy intake across physiological ranges of 0.3 to 0.9 pmol/kg/min infusions in healthy adults. Gutzwiller and colleagues (1999) identified a dose dependent reduction in energy intake following 0.3, 0.75 and 1.5 pmol/kg/min GLP-1 infusions, and early fullness and less hunger reported during infusions using the highest dose only. There was a reduction of around 280 kcal following 0.9 pmol/kg/min infusions of GLP-1 in healthy males, which translated into a 16% energy intake reduction when compared to the saline control in a study by Degen et al. (2006). Another study by Gutzwiller et al. (2004) also reduced caloric intake following 0.9 pmol/kg/min in healthy males, with an eating-inhibitory effect of 155 kcal compared to control. Interestingly, in the same study, CCK infusions were also identified to reduce caloric intakes, yet there was neither individual nor interactive effects on meal size or calorie consumption during simultaneous infusion of GLP-1 and CCK. There was however a significant decrease in subjective hunger ratings following infusion of both peptides. Combined infusion of these peptides reduced food consumption and calorie intake significantly less than the sum of their individual effects. This possibly occurred because both CCK and GLP-1 exert inhibitory effects on GER and these effects may at least in part be responsible for their satiety producing properties. However, Brennan et al. (2005) report no effect of synergism following GLP-1 and CCK administration in healthy adults, yet there is not enough evidence and the interaction between these two peptides is unclear. It is therefore unknown whether combined infusion of CCK plus GLP-1 exerts additional effects on GE or whether the effect of the combined infusion is similar to the effect induced by a single infusion of one of the two peptides. However, it is known that inhibition of GE and inhibition of food intake can be independent actions.

It is possible that testing conditions may affect GLP-1 satiation. Following a dose of 0.9 pmol/kg/min GLP-1 in healthy males, there was no inhibitory effect on eating reported in a study by Brennan et al. (2005), however supraphysiological doses between 1.2-1.5 pmol/kg/min GLP-1 reduced meal size in a study by

Gutzwiller et al. (1999). This was not supported by Long et al. (1999), who reported no effect on energy intake following 1.2 pmol/kg/min GLP-1 infusions on *ad libitum* eating. The discrepancies in eating effects between these three studies may be a result of study design. For example, *ad libitum* meals were not the same across studies. Sandwiches were offered in the study by Gutzwiller et al. (1999), whereas a selection of fruits, breads and cold meats were offered *ad libitum* by Brennan et al. (2005). In addition, all three studies took part on different times of the day, i.e., morning (Brennan et al. 2005), noon (Gutzwiller et al. 1999), and evening (Long et al. 1999).

There is evidence to suggest that GLP-1 acts in the abdomen to induce an eating-inhibitory effect. This is apparent from research conducted in patients who have had vagotomy and pyloroplasty procedures. Signalling via the vagus nerve was investigated in 20 truncally vagotomized subjects with pyloroplasty and matched with 10 healthy controls in a study by Plamboeck et al. (2013). Subjects received 1.2 pmol/kg/min GLP-1 (7-36 amide) or saline infusions during and after a standardized liquid mixed meal and a subsequent *ad libitum* meal. Despite no effect on appetite sensations, GLP-1 significantly reduced *ad libitum* food intake in the control group but had no effect in the vagotomized group. Gastric emptying was accelerated in the vagotomized subjects and was decreased by GLP-1 in controls but not in vagotomized subjects. Peak postprandial GLP-1 levels were approximately five-fold higher in the vagotomized subjects. These findings suggest that vagotomy with pyloroplasty impairs the effects of exogenous GLP-1 on food intake and GE, signifying that intact vagal innervation may be important for GLP-1's actions. Data from rat models also support a vagal mechanism. Recent findings by Krieger et al. (2016), who used a novel bilateral nodose ganglia injection technique to deliver a lentiviral vector and to knock down vagal afferent neuron (VAN) GLP-1Rs in male Sprague Dawley rats, report that VAN GLP-1R knockdown rats had increased meal size and accelerated GE. This is also supported by an earlier study by Rüttimann and colleagues (2009), who report GLP-1 infusions of 1.0 nmol/kg/min GLP-1 reduced meal size in rats with subdiaphragmatic vagal deafferentations. Both of these studies highlight a crucial role for the vagal afferents in mediating the effects of endogenous GLP-1 on food intake.

There is also evidence from animal models to support the role of intestinal GLP-1 acting locally to inhibit eating in mice and rats. First, the cranial mesenteric artery, which supplies much of the small and large intestines, may play a key role in the GI site(s) of action of GLP-1 on food intake. Infusion of 0.5 nmol/kg of GLP-1 in rats reduced meal size when infusions were via the cranial mesenteric artery. No reductions in meal size occurred when the infusion site of GLP-1 was the HPV or femoral artery (Williams et al. 2016). Second, injection of GLP-1-albumin conjugate, which is unlikely to cross the BBB, into the peritoneum of mice reduced energy intake (Baggio et al. 2004). Third, in chow-fed rats, GLP-1R antagonists administered intravenously have been reported to have no effect on eating (Kim et al. 2009a; Ruttimann et al. 2010; Williams et al. 2016) yet GLP-1R antagonists administered via the peritoneum have reduced eating in some studies (Williams et al. 2009; Williams et al. 2011) but not all (Abegg et al. 2013; Williams et al. 2016). The discrepancies between studies who have compared the effects of eating following GLP-1R antagonism via different sampling sites suggests that locally acting GLP-1 may only exert its effect in rats under certain conditions only. For example, the dietary fat content of feeds should be considered, as consumption of lipids has been shown to suppress the eating-inhibitory effect of GLP-1 (Duca et al. 2013).

Rodent studies have also identified several brain sites where GLP-1 may act to induce an eating-inhibitory effect, such as the nucleus accumbens, ventral tegmental area and NTS. As suggested earlier, findings from animal studies suggest that GLP-1 does not increase systemically after meals in rats, and that intestinal GLP-1 does not control eating in rats from an endocrine perspective, therefore these sites are potentially physiologically stimulated by neuronal GLP-1 in rats (Rinaman 2010; Llewellyn-Smith et al. 2011). Conversely, postprandial GLP-1 levels are relatively high in the systemic circulation of humans, therefore it is likely that endocrine GLP-1 does affect these brain areas in humans because GLP-1 can enter the brain by simple diffusion (Kastin et al. 2002). Evidence from Hayes et al. (2009) and Vrang et al. (2003) indicate the possibility of GLP-1 signalling via the NTS to influence satiation by modulating signals related to gastric distention. These studies highlight that gastric distension-induced endogenous GLP-1R activity in NTS neurons may contribute to the normal control of feeding and should be further investigated to identify whether there are endocrine involvements.

It is also possible that GLP-1 affects eating in other ways. As previously mentioned, feeding is clearly influenced by hedonic, reward-related factors, i.e., palatability, motivation, and learned associations and cues that predict the availability of food. Different neural circuits have been proposed to mediate these homeostatic and hedonic aspects of eating. The influence of GLP-1 on neural pathways that appear to be involved in integrating GI satiation signalling with food reward has shown that GLP-1 may contribute to flavour hedonics in rats (Mietlicki-Baase et al. 2014). Glucagon-like peptide-1 projections from the NTS to the nucleus accumbens and ventral tegmental area are suggested to play a role in mechanisms that may influence palatability, motivation for food and meal size (Williams et al. 2014). Dickson et al. (2012) report that GLP-1 agonism with exendin-4 reduced food-reward behaviour in rats following conditioned place preference testing, whereby rats who were administered with exendin-4 no longer preferred an environment previously paired to chocolate pellets and also showed decreased motivated behaviour for sucrose in a progressive ratio operant-conditioning paradigm when administered peripherally. Dickson et al. (2012) suggest these effects are mediated centrally, via GLP-1Rs, located in several key nodes of the mesolimbic reward system. In order to study the effects of GLP-1 receptor activation (independent of hormonal or metabolic changes induced by GLP-1 receptor activation) the somatostatin pancreatic-pituitary clamp technique can be applied in the clinical setting. The somatostatin pancreatic-pituitary clamp technique involves suppression of endogenous insulin, glucagon, growth hormone and GLP-1 production by administering somatostatin, thus allows identification of the contribution of individual counterregulatory hormones to defend against hypoglycaemia by creating isolated deficiencies of particular hormones. This technique was performed to investigate the acute effects of IV exenatide, with or without prior GLP-1 receptor blockade with IV exendin 9-39, on food intake and CNS responses to visual food cues using fMRI in human subjects by van Bloemendaal and colleagues (2014). Increased brain responses to food pictures in appetite and reward-related brain regions (insula and amygdala) were reported, and when compared to the placebo, exenatide decreased food intake and food-related brain areas. Although the effect is not definitive, evidence from both rodents and humans suggests GLP-1 has the potential to influence hedonic eating by influencing brain areas related to the production of hedonic judgements, thereby providing novel

insights into the mechanisms by which GLP-1 regulates food intake and how GLP-1 receptor agonists cause weight loss.

From a fluid and sodium perspective, GLP-1 may also have a physiological influence in regard to fluid intake. Rats injected with the antagonist exendin-9 were reported to drink more fluid in response to either subcutaneous hypertonic saline or water deprivation with partial rehydration when compared to rats who were vehicle-treated only (McKay et al. 2014). A study by Gutzwiller et al. (2006) highlighted the involvement of GLP-1 in sodium and water homeostasis also in humans. An IV salt load of 26.7 ± 0.9 g was given to healthy adults to compare the effect of an infusion of GLP-1 (1.5 pmol/kg/min) versus isotonic saline and it was shown that GLP-1 increased sodium excretion by the kidneys to control extracellular volume expansion.

With regards to animals and humans, it is important to highlight that there are two distinctions between chronic GLP-1 treatments and its eating-inhibitory effects between these species. First of all, the site of action in humans is unidentified (Steinert et al. 2017). For example, in rats who received GLP-1 agonist treatments to induce weight loss, there were central acting effects (Secher et al. 2014), but in humans who received liraglutide (GLP-1 agonist), there was low uptake of liraglutide in fluid sampled from the CNS (Christensen et al. 2015). Secondly, central administration of GLP-1 agonists can elicit symptoms of visceral illness (i.e., anorexia) in animal studies (Kanoski et al. 2012), however this is not a serious side effect in humans (Pi-Sunyer et al. 2015). These differences between animal and human studies highlights the need for a better understanding of these factors if GLP-1 is to be used as a pharmacological anti-obesity agent.

2.5.5 GLP-1 and Glycaemic Control

Insulin secretion, inhibition of glucagon secretion, slowing of GE and reductions in hepatic glucose metabolism are achieved by GLP-1 to control meal-related glycaemia (Campbell et al. 2013; Sandoval et al. 2015). There is also evidence to support the role of GLP-1 in contributing to glycaemic control in the

fasted state. The effect of GLP-1 on glucose appearance and glucose disposal was measured in healthy men who were subjected to the pancreatic clamp technique, with insulin and glucagon infused at rates adjusted to maintain blood glucose near fasting levels. Prigeon et al. (2003) report that concentrations of insulin, C-peptide and glucagon were similar before and during the GLP-1 infusion, and following the initiation of GLP-1 infusion, plasma glucose decreased in all subjects from steady-state levels of 4.8 ± 0.2 mmol/L to as low as 4.1 ± 0.2 mmol/L. This has also been shown in subjects with T2DM (Seghieri et al. 2013), suggesting that GLP-1 reduces endogenous glucose production during the fasted state in subjects with and without metabolic disease. Mouse models suggest that these effects may be achieved by an insulin-dependent effect on the GLP-1R in the HPV or liver (Burcelin et al. 2001).

Together with GIP, GLP-1 mediates the incretin effect by exerting dose-related, glucose-dependent insulinotropic effects on pancreatic β -cells (Drucker et al. 2006). When GLP-1 is infused in physiological endocrine doses (~ 0.33 pmol/kg/min) insulin secretion is increased in subjects who are fasted and in subjects receiving glucose infusions (Nauck et al. 1993; Vilsbøll et al. 2003). Additionally, in the study by Nauck et al. (1993), a 0.3 pmol/kg/min infusion of GLP-1 during isoglycaemic glucose administration (infusions of glucose which lead to the same glycaemic profiles as oral glucose), reproduced the same insulin response as oral glucose did. These studies provide evidence for GLP-1 to meet criterion 3 in **Table 1**, as such exogenous administration of GLP-1 in amounts duplicating the meal-related changes in endogenous patterns at the site of action produces a physiological endocrine incretin effect. Antagonism by exendin (9-39) has been shown to decrease insulin secretion after meals (Nicolaus et al. 2011) and after oral glucose loads (Salehi et al. 2008), during intraduodenal glucose infusions (Shirra et al. 2006) and whilst undergoing hyperglycaemic glucose clamps, all of which positively contributes to substantiation of GLP-1 to exert incretin effects, and thus meeting criterion 6, **Table 1**.

As mentioned, GLP-1 synergizes with GIP to enhance insulin release, however there is evidence to suggest that both incretins are not equipotent. The effects of intraduodenal glucose infusions at 2 and 4 kcal/min on plasma GIP, GLP-1 and glucose was investigated by Marathe et al. (2014), who highlight that the rate of

small intestinal glucose exposure (i.e., glucose load) is a major determinant of the comparative secretion of GIP and GLP-1. They indicate that GIP was the predominant incretin during glucose infusion of ≤ 2 kcal/min, whereas GLP-1 predominated during glucose infusions of either 3 or 4 kcal/min. Trahair and colleagues (2012) also show that following 3 kcal/min glucose infusions, GLP-1 release was more dominant than GIP. Both of these studies investigated incretin responses using intraduodenal glucose infusions up to 4 kcal/min, which is physiologically relevant because glucose usually empties from the stomach in a tightly regulated fashion at an overall rate of 1–4 kcal/min (Trahair et al. 2012).

Despite glucose stimulated-GLP-1 and GIP secretion normally reported in patients with T2DM (Ma et al. 2012; Calanna et al. 2013), the incretin effect is lessened (Drucker et al. 2006; Bagger et al. 2011). The most likely explanation for this is a decrease in pancreatic β -cell response to GLP-1 and GIP. As a consequence, GLP-1 is widely accepted to contribute somewhat more to the incretin effect in individuals with T2DM than healthy cohorts (Woerle et al. 2012). GLP-1 agonists have great potential for the treatment of T2DM. For example, before and after insulin treatment for four weeks in patients with T2DM, patients underwent three hyperglycaemic clamps with infusion of GLP-1, GIP or saline to compare insulin responses to incretins in a study by Højberg et al. (2009). Following insulin treatment, C-peptide responses increased significantly during GIP infusion and during GLP-1 infusion, highlighting that normalisation of blood glucose for 4 weeks improves β -cell responsiveness to both GLP-1 and GIP.

As previously discussed, GLP-1 slows GE, which in turn improves glycaemic control (Witte et al. 2009). With regards to healthy subjects, the diverse effects on glycaemia following GLP-1 infusions suggest that glucagonostatic and gastric inhibitory effects outweigh the insulinotropic effect (Nauck et al. 1997; Nicolaus et al. 2011). The effects of GLP-1 on gut motility are pertinent for the treatment of T2DM. For example, a deceleration of GE by GLP-1 has been demonstrated in patients with T2DM. Nauck et al. (2011) report that following GLP-1 antagonism to slow GE, postprandial glycaemia and insulin secretion is largely diminished when the effects of the incretin on the stomach are antagonized. This highlights the importance

of delayed GE as a primary factor in driving reductions in postprandial glycaemia during acute GLP-1 administration, factors of which are of ultimate value for conditions of impaired glucose handling.

Figure 4 summarizes the GI physiology of GLP-1 discussed in this section, highlighting both well established and less established effects of the peptide with solid and dashed lines, respectively.

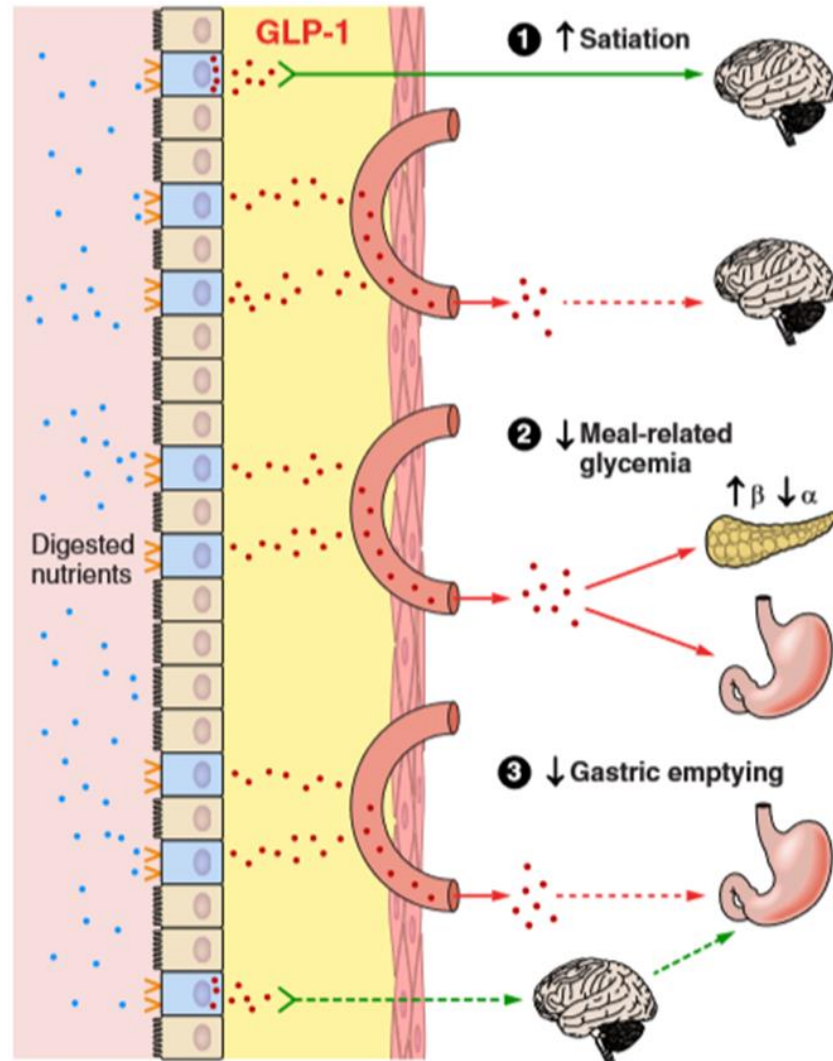


Figure 4. GLP-1 physiology. 1) GLP-1 stimulates satiation via vagal afferents (green arrow). Data in rats indicate GLP-1 signals satiation via a local action on vagal afferents (green arrow). GLP-1 may also act in the brain to affect satiation or postprandial satiety. 2) GLP-1 improves meal-related glycaemia by increasing pancreatic β -cell insulin secretion in a glucose-dependent manner, by inhibiting pancreatic β -cell glucagon secretion, and by inhibiting gastric emptying; all three appear to be endocrine effects of GLP-1. 3) GLP-1 slows gastric emptying via a direct endocrine effect and perhaps via a vagal-vagal reflex. Diagram taken from Steinert et al. (2017).

In summary, GLP-1 delays GE and gut motility in humans, influencing nutrient delivery to the small intestine. Peripherally administered GLP-1 also affects the central regulation of feeding in both animal and human studies. It is therefore the synergistic actions of GLP-1 in the gut and brain, acting on both central and peripheral receptors, that appear responsible for the effects of the peptide hormone on appetite mechanisms. Yet, although it possible to measure GLP-1 as a biomarker of appetite, assessment of appetite itself is not so straightforward. The following section discusses pitfalls in quantifying subjective feelings related to appetite.

2.6 Appetite: Is accurate evaluation possible?

It is not unusual to say, “I am full” and then proceed to consume more food. Neither is it uncommon to express that one is “hungry” when that hunger is not accompanied by the intense uncomfortable sensations in the stomach that characterize hunger pangs. With regards to hunger signals, Bernstein and Santos (2018) recently suggest that overweight individuals are not aware of subtle cues that allow the termination of an eating episode once satiation is reached. In their study, overweight individuals required the presence of stomach growling or pain to indicate hunger, and additionally relied on feelings of discomfort to signify fullness. Murray and Vickers (2009) explored the complex ideas of hunger and fullness in groups of normal weight and overweight dieting and non-dieting females. Within these groups, hunger and fullness sensations were described as having both physical and psychological components that were divided into two groups: ‘typical’ and ‘extreme’. Overall, hunger was described as the presence of stomach growls, stomach hunger pains, emptiness, focus on eating, loss of energy, and desire to eat. Fullness was described as a feeling of food in the stomach, stomach stretch, satisfaction, contentment, energized, focused, and lack of the desire to eat. Typical fullness was described with many psychological components while typical hunger was primarily physical in nature. Participants described situations in which sensations of hunger and fullness overlapped, which provides evidence that hunger and fullness are not polar opposites. This qualitative research suggests that individuals can have diverse views of what constitutes hunger and fullness, and these views are not detailed enough to capture physical and psychological aspects.

Post-prandial hunger and fullness ratings do not always translate into eating less, nor do they foretell future weight loss, which is clearly dependent on diet and exercise. A single meal or food ingredient may have a significant effect on satiety when eaten on its own or part of a particular food matrix (Rebello et al. 2013; Rebello et al. 2014). A satiety response is dependent on individual responsiveness to physiological signals that are competing with environmental cues to eat. It may also not be possible for individuals to incorporate the food into their normal eating patterns. Therefore, the satiety enhancing food may deliver the claimed benefits within an explicit behavioural or usage context. Despite this, in a systematic review by De Graaf and colleagues (2004) that included almost 80 studies, appetite was assessed by rating subjective feelings of appetite or by assessing food intake in a standardized setting. In almost all of the studies included, these measures were in line with each other, i.e., lower appetite ratings correlated with a lower food intake in a standardized setting. The validity of the rating scales and food intake assessment are reinforced by this observation, and can be used as stand-alone measure of evaluating appetite.

For a satiety claim to be valid, it must deliver an enhanced satiety in comparison to a control meal/product on repeated exposure. A multi-step proof-of-concept examination is required to identify whether or not a single product will lead to sustained satiety, and therefore weight loss. Learned satiety suggests that associations between the sensory properties of food and the post-ingestive effects result in an acquired control of meal size (Booth 1977).

Even though a product may not have lasting benefits in relation to promoting satiety, there may be short-term advantages for the consumer. For example, aiding compliance with energy restriction diets or perhaps making the weight loss experience more enjoyable than it would be otherwise are possible outcomes that can contribute to achieving behavioural goals such as managing weight or training eating habits that are beneficial to health. Evaluating the motivation to eat offers useful insights that aid development of interventions targeting eating behaviour that may alter energy balance in the desired direction (Hetherington et al. 2013).

2.6.1 Beyond Satiety: Scientific Considerations

Given the plethora of research related to determinants of satiety, it is rational to consider the evidence for the potential impacts this could have. Food companies are challenged to justify that foods carrying substantiated satiety claims deliver a benefit to the consumer, particularly if only acute behavioural effects have been demonstrated. Foods that increase fullness and help to control hunger have the possibility to provide benefits to the consumer in several different ways, including those related to longer-term weight management. However, Blundell (2010) warns that: *‘The wording of a claim is, therefore, critical. The difference between a proof of concept and a guarantee of success is an important point that needs to be conveyed to the consumer.’* Components of foods that support satiety signals may have clear corroboration from laboratory-based studies in the short term, but there is uncertainty over whether and how enhancing satiety effects of foods as a general principle translates to physiological or health benefits to the consumer, such as in managing hunger or maintaining a healthy diet (Hetherington et al. 2013). According to Hetherington and colleagues (2013), it is reasonable to suggest possible benefits to the consumer of foods that enhance satiety, which include the following:

- (a) Providing appetite control strategies for consumers who are highly responsive to the food environment and eat opportunistically;
- (b) Offering pleasure and satisfaction associated with low-energy/healthier versions of food products without feeling ‘deprived’;
- (c) Reducing dysphoric mood states associated with feeling hungry especially during periods of energy restriction;
- (d) Increasing feelings of subjective wellbeing, maintaining or preventing a decline in cognitive function associated with skipping meals or lowered energy intakes;
- (e) Improving the ability to cope with fluctuations in hunger over the day;
- (f) Improving compliance with healthy eating targets and weight-management efforts;

(g) Increasing self-efficacy through adherence to diet goals;

(h) Achieving weight loss and preventing weight (re)gain, maintaining a healthy body weight and reducing risk of weight regain.

(Hetherington et al. 2013)

From these suggestions, it is clear to see that the benefits offered by enhancing satiety are diverse, ranging from short-term considerations (i.e., early termination of a meal) to long-term (i.e., helping consumers reach their dietary goals). **Figure 5** shows the possible routes to end-benefits from incorporating individual satiating foods within a dietary pattern. An important concept is that beyond the simple notion that enhancing satiety can benefit consumers directly by decreasing energy intake, various other indirect routes, which may be beneficial to the consumer, are also possible and perhaps may be more achievable. For example, increasing general dietary control, or improved success in meeting weight management goals. Amongst the many benefits of increasing satiety, a principle benefit may be assisting weight loss, since subjective hunger ratings have been shown to predict weight loss failure in clinical trials (Womble et al. 2001). Giving consumers guidance regarding dietary patterns and products that offer pleasure, satisfaction and a potential way to manage hunger could be of benefit. Foods can be chosen, and/or their characteristics altered to promote satiation within a meal, i.e., increasing the fibre content of a food or drink. Yet there is a considerable gap in knowledge between evidence that is gathered under experimental conditions and claims made about the enhancement of satiety in the context of day-to-day lives of the consumer.

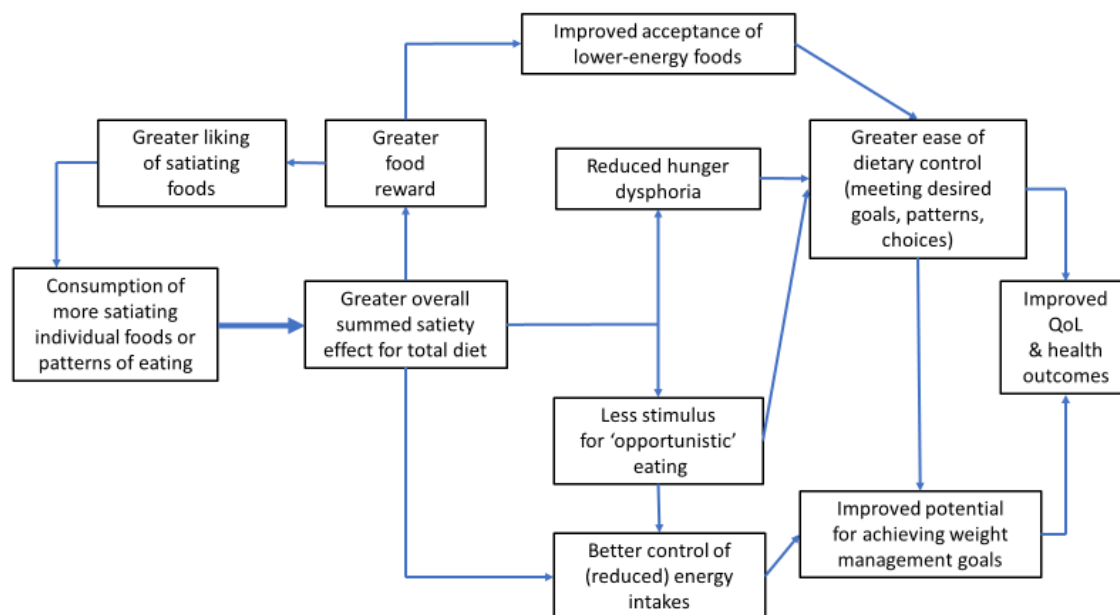


Figure 5. Schematic illustration of the potential routes to end-benefits from incorporation of more satiating individual foods within an overall dietary pattern (Diagram taken from Hetherington et al. 2013).

2.7 Dietary fibre definitions and current intakes

A number of analytical methods for dietary fibre have been used in the UK over the years for the purpose of nutrition labelling, and these methods have changed as the definition of dietary fibre has evolved. In the 1980s and 1990s, a method developed by Englyst (Englyst et al. 1995), which measures plant cell wall components of dietary fibre referred to as non-starch polysaccharides (NSP), was the recommended UK method for nutrition and labelling purposes (Hooper et al. 2015). Since 2000, the UK has adopted the AOAC method for fibre quantification, and it is such method that current dietary fibre recommendations are based on. The AOAC value includes lignin and resistant starches, which are not part of the NSP fraction, and for this reason, the method tends to result in fibre values approximately one third higher than those obtained using the Englyst method. The AOAC method is used globally, therefore the transition to this measurement in the UK makes it easier to compare intakes and recommendations across countries, which is particularly important for clinical studies investigating health benefits of dietary fibre.

The Scientific Advisory Committee on Nutrition (SACN 2015) has extended the definition of dietary fibre to include not only NSPs but also non-digestible oligosaccharides, resistant starch and polydextrose as there is now evidence to demonstrate similar beneficial physiological effects (e.g. stool bulking, decreasing intestinal transit time and constipation, or lowering total and LDL-cholesterol) to those demonstrated for naturally integrated (as opposed to extracted natural or synthetic) dietary fibre components of food. Therefore, the 'new' definition for fibre includes all carbohydrates that are neither digested nor absorbed in the small intestine and have a degree of polymerisation of three or more monomeric units, plus lignin. UK dietary fibre recommendations for adults advocate consuming 30 g of fibre per day (SACN 2015). Current intakes are around 10 g below the recommendation for men and 13 g below for women (NDNS 2016). Given current intakes, achieving this recommendation would require a 50% increase in fibre intake for men and a 75% increase for women. Around 80% of the Scottish population are still not consuming five portions of fruit and vegetables each day (SHeS 2017). It is therefore suggested that innovative high-fibre ingredients may make it easier for consumers to boost their fibre intakes, alongside clear information about the benefits of fibre.

2.7.1 Oats and Oat Products

Oats were traditionally eaten as porridge or baked goods to which salt and/or sugar were added (Kapica 2001). However, modern product innovation has created a selection of oat products to meet the needs of a vast range of consumers. This has greatly improved the convenience and acceptability of oats and could help widen appeal to those who do not typically consume oats. New products include instant porridge, designed to be consumed 'on the go', along with mueslis and granolas. Oat breads are becoming more common, normally consisting of a combination of wheat flour and added oats (Rasane et al. 2015), while oatcakes have moved beyond the traditional type to include finely milled, rough, those flavoured with cheese or herbs and gluten free options. Consuming oats as porridge is not to everyone's taste, with poor cooking skills and time acting as barriers to porridge consumption (Ahmad et al. 2014). In light of this, new oat-based products have become available. The nutritional content can vary, in terms of sugar and salt

contents, however the positive contribution of fibre, particularly β -glucan, should be strongly considered given current European Food and Safety Administration (EFSA) health claims following consumption.

2.7.2 β -glucan

β -D-glucans, usually referred to as β -glucans, comprise a class of indigestible polysaccharides widely found in nature in sources such as grains, barley, yeast, bacteria, algae and mushrooms. In oats, they are concentrated in the bran, more precisely in the aleurone and sub-aleurone layer (**Figure 6, A**). Oat β -glucan is a natural soluble fibre and is a viscous polysaccharide made up of units of the monosaccharide D-glucose. Oat β -glucan is composed of mixed-linkage polysaccharides. This means the bonds between the D-glucose units are either β -(1 \rightarrow 3) linkages or β -(1 \rightarrow 4) linkages (**Figure 6, B**). This type of β -glucan is also referred to as a mixed-linkage (1 \rightarrow 3), (1 \rightarrow 4)- β -D-glucan. The (1 \rightarrow 3)-linkages break up the uniform structure of the β -D-glucan molecule and make it soluble and flexible (Wang and Ellis 2014).

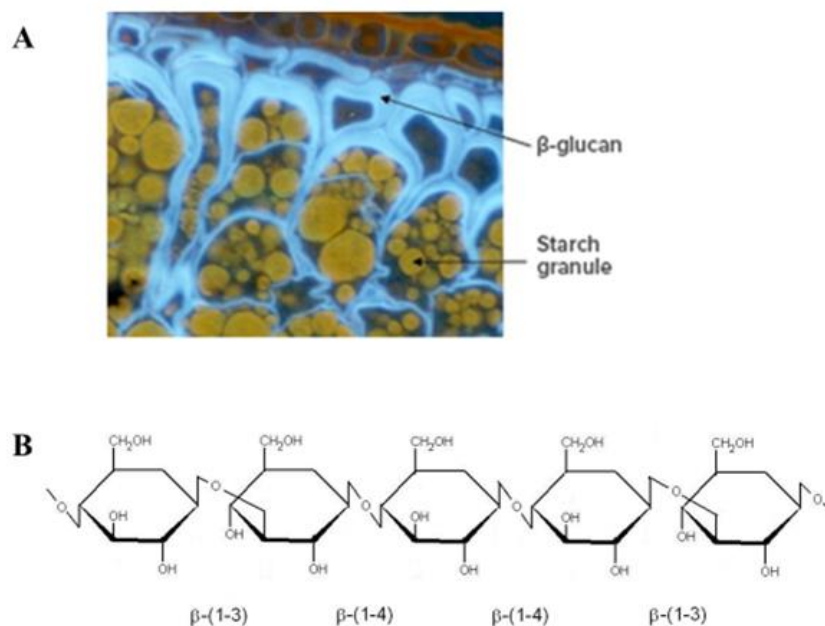


Figure 6. Oat grain stained with Calcofluor and Acid Fuchsin showing the location of β -glucan in aleurone and sub-aleurone layer of the oat (A) and chemical structure of oat β -glucan (B). Images taken from Wang and Ellis (2014).

2.7.3 Health Claims

Following the discovery of the bioactivity of cereal soluble fibre, (1→3,1→4)- β -D-glucan, there has been extensive attention among researchers, the food industry and consumers since the 1980s. Several authorities, including the U.S Food and Drug Administration (FDA) and European Food and Safety Administration (EFSA), have acknowledged the health benefits of β -glucan consumption by broadcasting ratified health claims based on scientific evidence associated with this specific soluble fibre. The generic health claim that β -glucan ‘may reduce the risk of heart disease’ was first issued in 1997 by the FDA given that 0.75 g of soluble β -glucan fibre is consumed per serving when a total of 3 g is consumed daily. Following this, numerous European authorities have pro-claimed the cholesterol lowering ability of β -glucan (United Kingdom Joint Health Claims Initiative (JHCI; 2006); Agence Française de Sécurité Sanitaire des Aliments (AFSSA 2008; EFSA 2009)). A more recent opinion issued by EFSA (2011) states that ‘*Oat beta-glucan has been shown to lower/reduce blood cholesterol. Blood cholesterol lowering may reduce the risk of (coronary) heart disease*’ when at least 3 g of oat β -glucan is consumed per day as part of a balanced diet. As mentioned, besides the attenuation of cholesterol, β -glucan also plays a role in lowering postprandial blood glucose and insulin responses. In order to obtain the claimed effect EFSA (2011) state that ‘*4 g of β -glucans from oats or barley for each 30 g of available carbohydrate should be consumed per meal*’, thus β -glucan consumption is of interest in those who wish to improve their CV health. Several attempts have been made by food companies to attain a health claim based on increasing satiety following the consumption of β -glucan, however despite the scientific evidence that β -glucan potentially ‘increases satiety, prolongs satiety’ there have been no official EFSA or FDA health claims issued. Although EFSA recognize that an increase in satiety leads to a reduction in food intake, and if sustained may be of a beneficial physiological effect, they highlight that a direct ‘*cause and effect relationship has not yet been established between the consumption of β -glucans from oats and barley and a sustained increase in satiety leading to a reduction in energy intake*’. The influence of β -glucan on appetite control is still insufficiently characterized and warrants further investigation.

2.8 β -glucan and satiety

β -glucan has been shown to increase satiety-related hormones as well as reduce energy intake and body weight in animal models. Huang and colleagues (2011) report that β -glucan from oats activated the gut-hypothalamic (PYY3–36-NPY) axis, satiety, and weight loss in diet-induced obese mice with a satiety test. Additionally, the highest β -glucan diet (7 g β -glucan per 100 g of feed) significantly increased plasma PYY(3–36), with suppression of ARC NPY mRNA. As with other animal studies discussed, results reported in animal models may not be entirely transferable to humans. With regards to the study by Huang et al. (2011), 7 g β -glucan per 100 g of feed is an extremely high dose of β -glucan for mouse ingestion, therefore results should be interpreted with caution. Nevertheless, further investigation by Lin et al. (2013), who investigated a dose-dependent effect of oat cereal β -glucan on improving metabolic indexes of obese mice using three doses of β -glucan, has been performed. Oral doses of low 0.5 g/kg/bw, medium 1 g/kg/bw and high 1.5 g/kg/bw β -glucan were administered for three weeks, with negative correlations found between body weight change and average energy intake with β -glucan concentration. Both ARC NPY mRNA and ARC neural peptide Y receptor 2 mRNA levels were negatively related to β -glucan concentration, while plasma NPY was positively related to different β -glucan doses, thus increasing the dose of β -glucan reduced energy intake and body weight, yet the same relationship was not identified for PYY, as dose change of β -glucan resulted in varied plasma NPY and NPY mRNA. Adam et al. (2014) assessed the impact of feeding young adult male rats an *ad libitum* diet containing 10% w/w oat β -glucan for four weeks on satiety hormone secretion and body composition. Following the study period, plasma GLP-1 and PYY increased by 2.2 and 2.3-fold, respectively. In addition, a 10% reduction in food intake and 37% reduction in body weight accompanied by a 26% loss of body fat were reported in male rats that consumed oat β -glucan. Again with reference to animal to human interpretation, although findings in this study are promising, it is important to highlight that the amounts of soluble fibre consumed throughout the intervention period demonstrates an animal model of fibre satiety, however the amounts of fibre consumed (6.6 mg/kJ) was approximately twice

the daily fibre intake of 3.3 mg/kJ for men (Turner and Lupton 2011), thus further work is required to translate the findings into humans.

Human studies evaluating the effects of oat β -glucan on satiety have mostly focussed on evaluating acute effects (<24 hours), and tested whole foods as well as β -glucan extracts added to food products. A summary of acute energy intake and appetite studies following cereal β -glucan consumption can be found in **Appendix 1** and are discussed in detail throughout this section.

There are several studies that report an eating-inhibitory effect following β -glucan consumption, with doses ranging from 2.68 g to 5.65 g (Beck et al. 2009a; Rebello et al. 2016a). Modest reductions in energy intake subsequent to β -glucan consumption have been reported to be within a range of 85 kcal to 96 kcal (Rebello et al. 2016a; Beck et al. 2009a), with larger reductions of up to 244 kcal documented (Geliebter et al. 2015). A concentrated barley β -glucan extract was used by Vitaglione and colleagues (2009) to enrich bread that was used as a vehicle to deliver 3 g β -glucan at breakfast to a group of 14 healthy normal weight subjects. Following consumption of the β -glucan-enriched bread, energy intake reduced by 172 kcal at an *ad libitum* lunch served 3 hours following test bread consumption compared to a control bread containing 0 g β -glucan. Hunger sensations were significantly reduced following the β -glucan-enriched bread, along with increased sensations of fullness 2-3 hours following test bread consumption. Satiety was also increased 2 hours following consumption of the β -glucan-enriched bread. The study also investigated plasma glucose, ghrelin and PYY responses, and reported that β -glucan bread reduced glycaemia and ghrelin and increased PYY responses in the short term more effectively than the bread with no β -glucan. Associations between ghrelin, PYY and subjective appetite ratings were reported following consumption of the β -glucan bread, which is suggested to have influenced the reduction in *ad libitum* eating. More recently, Geliebter et al. (2015) reported a decrease in 244 kcal following 4 g of oat β -glucan in both normal and overweight subjects. Subjects consumed either oatmeal (4 g β -glucan), frosted flakes (0 g β -glucan) or a water control at breakfast, followed by an *ad libitum* lunch 3 hours later. Following subgroup analysis, it was shown that the eating-inhibitory effect was more pronounced in

overweight subjects following the oatmeal breakfast than normal weight subjects who consumed 357 kcal and 742 kcal, respectively. There was also a positive effect reported on fullness and hunger after oatmeal consumption when compared to both frosted flakes and water. There were no differences in glucose or insulin responses between groups, despite slower GE following the oatmeal breakfast. Thus, greater satiety after the oatmeal breakfast may have been due, in part, to slower GE, which can result in prolonged gastric distention. Although GE can also be delayed by increasing the energy value of a meal (Calbet et al. 1997) the two cereal breakfasts had the same energy values (351 kcal), suggesting that GE was slowed by other factors, such as the β -glucan content of the oatmeal. The sugar content of the breakfasts differed, with the oatmeal containing 8.3 g and frosted flakes 35.5 g per serving. Since there were no significant differences in postprandial glycaemia, it can be suggested that the effects on satiety were independent of glucose. Despite the results from this study reporting a reasonable subsequent energy deficit, it should be noted that the study by Geliebter et al. (2015) is the only study to investigate an eating-inhibitory effect of β -glucan using a liquid *ad libitum* test meal. A nutritionally complete formula was offered to subjects to drink *ad libitum* (1 kcal/mL of their preferred chosen flavour). It is suggested that individuals will consume more *ad libitum* from liquid foods than they consume of solid foods, which is linked to the rate of eating being greater for liquid meals (Zijlstra et al. 2008; Blundell et al. 2010). The higher eating rate may be caused by the bite/swallow size, which is higher in liquids. For example, eating 500 g of apples takes around 17 minutes, whereas the time taken to drink the equivalent amount is achieved in around 1.5 minutes, according to Haber et al. (1977). Although slower eating does not necessarily lead to lower food intake (Martin et al. 2007), it is probable that subjects could have easily consumed > 500 g juiced apples without hedonic factors such as SSS influencing their intake. On the contrary, it was reported that subjects consumed around 357 kcal at the *ad libitum* lunch in the investigation by Geliebter et al. (2015), which in comparison to other studies that used solid *ad libitum* test foods, can be considered moderate given that subsequent energy intakes were reported to be around 700 kcal in studies investigating the same amount of β -glucan (Hartvigsten et al. 2014; Korczak et al. 2014). Therefore, it should be considered that perhaps the liquid test meal may not have given a true

representation of subsequent energy intakes by Geliebter and colleagues, and challenges arise when making comparisons across different studies that have used solid *ad libitum* test meals. When the same breakfasts were consumed daily by overweight subjects over a period of four weeks, the control group who did not consume breakfast lost more weight (-1.8 kg) compared to the groups consuming either frosted flakes or oatmeal (Geliebter et al. 2014). There was no difference in bodyweight between both breakfast groups, despite the oatmeal group reporting increased satiety. However, the method used to measure subjective appetite was not by traditional 100 mm visual analogue scales (VAS) and instead ratings of hunger and fullness were obtained using a Likert-type rating scale. Subjects were asked to select the number which best reflected their current hunger and fullness based on a line with anchor points at: 0, not at all; 20, slightly; 40, moderately; 60, quite; 80, very; and 100, extremely. Thus, this scale was not continuous and has unknown magnitudes of satiety at equally spaced intervals along the scale.

It is suggested by Beck et al. (2009a) that the optimal dose of β -glucan lies between 4 and 6 g to have a positive effect on appetite ratings and energy intake. A reduction in energy intake by approximately 96 kcal at an *ad libitum* lunch served 4 hours following a breakfast cereal containing 5.65 g β -glucan was reported by Beck et al (2009a), but no effect on *ad libitum* eating was seen following cereals containing 3.8 g or 5.45 g β -glucan. When compared to the control breakfast, a significant increase in subjective fullness ratings were reported for all breakfasts containing β -glucan, but were not dose-responsive. The breakfast containing 5.45 g β -glucan had the strongest effect on subjective appetite yet had no effect on energy intake. Positive effects on subjective appetite without translating into a reduction in physical eating have been reported elsewhere (Juvonen et al. 2009; Hartvigsten et al. 2014). In a crossover trial that involved subjects consuming a control wheat bread (0.2 g β -glucan, 1.1 g arabinoxylan), arabinoxylan (0.3 g β -glucan, 7.1 g arabinoxylan), β -glucan (4.2 g β -glucan, 2.6 g arabinoxylan) or rye kernel bread (1.5 g β -glucan, 6.1 g arabinoxylan), there was no eating-inhibitory effect of any test breads. However, reduced hunger and prospective food consumption along with increased satiety was reported following all three added fibre breads, with only the rye and arabinoxylan breads increasing fullness compared with the control bread. Neither breads had an impact on plasma ghrelin, but rye kernel bread and arabinoxylan breads increased

postprandial GLP-1 more than the β -glucan bread between 2-4 ½ hours after consumption (Hartvigsen et al. 2014). This study suggests that fibre enriched bread increases satiety with no effect on ghrelin concentrations or energy intake. Additionally, a small dose of β -glucan, 1.5 g, increased GLP-1 4-4 ½ hours following consumption. A similar effect of GLP-1 was also reported by Juvonen et al. (2009) following 5.1 g β -glucan consumed from a low viscosity test beverage. However, not only was the β -glucan dose greater in the study by Juvonen and colleagues (2009) but the beverage was enzymatically manipulated to lower viscosity. Although Hartvigsen et al. (2014) report that high MW β -glucan was used to enrich test breads, viscosity was not measured, thus it is not possible to identify whether the increase in GLP-1 occurred as a result of a specific dose or because of physiochemical characteristics of the test bread. In addition, arabinoxylans, composed of arabinose and xylose, are similar to β -glucan in that they are both classed as hemicellulose in nature, therefore findings from the study by Hartvigsen et al. (2014) should be interpreted with caution as β -glucan was consumed in conjunction with arabinoxylan in three out of the four test breads consumed.

There is evidence of no effect on acute appetite and energy intakes in acute studies that have used a variety of cereal β -glucan doses in test meals, ranging from 0.5 g (Hlebowicz et al. 2007) to 12.9g β -glucan (Clegg and Thondre 2014). When delivered in a semi-solid pudding, isocaloric servings (300 kcal) containing 1.5 g of dietary fibre, 10.3 g of insoluble fibre from wheat bran, 10.2 g of fibre from oat bran (5 g β -glucan) and a combination of wheat and oat bran providing 10.1 g fibre (2.5 g β -glucan), there was no effect on appetite ratings or energy intake following a subsequent lunch meal between all conditions tested in the 20 healthy normal weight subjects who completed the study. Despite a significant decrease in post-prandial glucose and insulin responses following the oat bran pudding, there was no effect on PYY and ghrelin across all conditions. When rated by subjects before eating, the meal with no added fibre was anticipated by subjects to be most filling out of all puddings (Juvonen et al. 2011). Cognitive factors, such as an estimation of the satiating effect of foods, contribute to making eating largely a learned behaviour (Blundell et al. 2010) and therefore could have influenced the results of this study, highlighting the complex nature of appetite and responses to meal manipulations. Furthermore, the physicochemical properties of the fibres

were not evaluated. A similar dose of β -glucan (approximately 6 g) was also reported to have no effect on energy intake. The study compared the effect of a small preload with and without β -glucan (150 kcal) and a large preload with and without β -glucan (450 kcal), incorporated into test biscuits. With regards to β -glucan content, the small and large preload delivered 2 g and 6 g, respectively. The β -glucan snacks were more effective in increasing fullness when compared to their caloric controls with 0 g β -glucan, however there were no significant differences in appetite parameters between the β -glucan biscuits (Vitaglione et al. 2010). Korczak and colleagues (2014) also report no effect on appetite or subsequent energy intake at an *ad libitum* lunch served 4 hours following consumption of breakfast bars varying in fibre content. Subjects received 10 g oat bran, 10 g barley bran and 3 g low fibre bars in a random order. Methane and hydrogen were also monitored in the study by breath testing over the 4 hour inter-meal interval. No differences were seen in either hydrogen or methane between the oat bran, barley or low fibre control, suggesting no enhancement of colonic fermentation. Yet the results may have been disadvantaged due to the duration of breath testing carried out. Bran fibres are heterogenous and may be fermented slowly, with reports of these fibres taking up to 24 hours to be fully digested by colonic bacteria (Peters et al. 2009). Additionally, the effects of bran fibres on appetite and energy intake might appear as early as six hours following consumption (Juvonen et al. 2009). It is also important to consider that the female subjects who participated in the study were low fibre consumers, with a reported 5 g of fibre intake per day.

As mentioned, doses of β -glucan investigated ranged from 0.5 g to 12.9 g, of which no effect was reported on appetite or energy intake. A 1.2 g barley meal replacement bar did not diminish hunger ratings or have an eating-inhibitory effect in 21 healthy overweight subjects (Peters et al. 2009). Similarly, no effects were seen by Clegg and Thondre (2014) following soups delivering 0 g, 3.61 g or 12.9 g barley β -glucan in healthy normal weight males. The MW of both fibre-containing soups were determined to be low MW and high MW, suggesting that differences in physiochemical properties of barley β -glucan did not influence subjective appetite or energy intake. Conversely, Lyly et al. (2010) report that a beverage containing 2.5 g and 5 g of β -glucan had a larger AUC for satiety and smaller AUC for hunger compared to a control beverage. Both β -glucan containing drinks were investigated with and without the addition of β -glucanase

to identify whether viscosity influenced appetite parameters. Low viscosity β -glucan (31.6 mPa.s) increased satiety compared to the control beverage but less compared to the beverage with the same amount of fibre with high viscosity (661 mPa.s). With regards to satiety ratings, when the amount of β -glucan was doubled from 2.5 g to 5 g in the 300 g beverage sample no dose-response relationship was reported. The beverage containing 2.5 g of β -glucan produced significantly higher ratings of satiety than the beverage without fibre. However, when the dose was raised to 5 g of β -glucan, although ratings were systematically higher, no significant difference was observed between the two quantities of dietary fibre levels (2.5 g and 5 g). The authors suggest that for achieving increased perceived satiety, the addition of 2.5 g β -glucan to a beverage is a sufficient amount. This study supports the hypothesis that viscosity is more potent in influencing satiety than actual dose of β -glucan available. Measurement of the physiochemical properties of the food matrix is often not carried out in studies investigating the satiating effect of cereal β -glucan (Juvonen et al. 2011; Korczak et al. 2014; Vitaglione et al. 2009; Vitaglione et al. 2010; Willis et al. 2009), which is a considerable limitation given that not enough is known from existing evidence to draw conclusions regarding the effectiveness of factors such as viscosity, MW and solubility on appetite and energy intake. Another factor that is poorly addressed in the literature is GE, with very few studies measuring stomach emptying following β -glucan consumption in relation to eating (Hlebowicz et al. 2007; Hlebowicz et al. 2008; Juvonen et al. 2009; Geliebter et al. 2015).

When delivered in semi-solid form yoghurt, no differences in satiety or GE were observed in a comparison between oat bran containing 4 g β -glucan and cornflakes (Hlebowicz et al. 2008). Using a similar design, Hlebowicz et al. (2007) also investigated the effects of breakfast cereals consisting of wheat bran flakes (7.5 g fibre), oat flakes (4 g total fibre of which 0.5 g β -glucan), and cornflakes (1.5 g fibre), of which similar results were also reported. In both studies by Hlebowicz and colleagues, GE was monitored by ultrasonography 15 and 90 minutes following test breakfast consumption. Neither a modest dose (4 g) or negligible dose (0.5 g) of β -glucan had any effect on GE, suggesting that β -glucan in a semi-solid meal does not affect stomach emptying or satiety in healthy individuals. Unlike the 0.5 g dose, there was however a significant decrease in postprandial blood glucose following 4 g β -glucan when compared to cornflakes,

suggesting that the attenuation of blood glucose was independent of GE. It is possible that a reduction in blood glucose occurred as a result of the viscosity of the digesta, acting as a physical barrier to glucose absorption kinetics in the GI tract, which is concurrent with recent findings. Wolever et al. (2018) recently report that to achieve a $\geq 20\%$ reduction in the AUC relative to a control meal of rice cream, 1.6 g of oat β -glucan was required to be consumed in oatmeal that already contained 1.2 g β -glucan, thus a net amount of 2.8 g β -glucan was efficient in attenuating post-prandial glucose responses in healthy individuals. There was no effect on subjective appetite ratings in both studies by Hlebowicz and colleagues, where satiety was assessed at 15 and 90 minutes only following the testmeals and by using a single numerical scale ranging from extreme hunger to extreme satiety punctuated with phrases describing various degrees of hunger and satiety. Monitoring and recording appetite ratings was not performed frequent enough or long enough to capture changes in appetite ratings as a traditional 100 mm VAS administered up to twice per hour would detect. Additionally, physical eating was not measured in both studies, therefore it is not possible to draw conclusions with regards to the effect of GE on eating and appetite sensations despite Hlebowicz and colleagues using a gold-standard method to investigate GER. As mentioned, Geliebter et al. (2015) report that GE was significantly slower following a semi-solid oatmeal breakfast containing 4 g β -glucan, with increased satiety accompanied by an eating-inhibitory effect of 244 kcal when compared to frosted flakes and a water control. However, there was no effect on eating following 5.1 g containing beverages with low and high viscosity, despite the GER of the high viscosity beverage being significantly faster than the low viscosity beverage (Juvonen et al. 2009). The low viscosity drink increased satiety more, and increased post-prandial PYY, CCK and GLP-1 were also reported. This study highlights the complexity of physiochemical factors and their role in appetite. Viscosity differences in oat β -glucan in a liquid meal with identical chemical composition strongly influenced short-term gut peptide responses and GER, implying the importance of food structure in the modulation of post-prandial satiety related physiology. There is a lack of evidence to make any firm conclusions regarding the relationship between β -glucan, GER and energy intake, thus robust studies focussing on such parameters in relation to the physiochemical properties of the test food matrix are warranted.

In the very few studies discussed that have measured GER along with subjective appetite and/or energy intake, differences in methods to assess such parameters are varied, i.e., using paracetamol and sonography for monitoring GE, and using 100 mm VAS for appetite ratings. Differences in methodological techniques is not only exclusive to the studies investigating the effect of β -glucan on GE and appetite, but across acute appetite studies as a whole.

2.8.1 Physicochemical properties of fibre

It is suggested that inhibition of subjective appetite, energy intake and body weight by fibre-rich diets may depend on the chemical structure of fibre and its physicochemical properties rather than the actual quantity of fibre consumed (Slavin and Green 2007; Wanders et al. 2011). The physicochemical properties of fibres associated with appetite, energy intake and body weight include solubility, viscosity, water-holding capacity and fermentability (Potty 1996; Wanders et al. 2011). Such properties may not only affect the satiating capacity of dietary fibres, but could also impact long-term appetite and consequently the regulation of energy intake.

Dietary fibres may affect appetite and energy intake directly via physical effects in the GI tract, i.e., viscosity. Viscosity of a fluid is described as resistance to flow, and despite the terms viscosity and gelling often being used interchangeably, their properties differ. A gel does not flow but instead stretches (elastic-like) or breaks under a force. The viscous nature of fibre favours appetite by several proposed mechanisms. Because viscous dietary fibres have the capacity to hold large quantities of water, they can increase stomach distension, in turn triggering afferent vagal signals of fullness (De Graaf et al. 2004). From a sensory perspective, a viscous bolus may increase satiety by increasing oral cavity exposure time. Gastric emptying is also potentially reduced by a viscous bolus, thus prolonging the absorption of nutrients (Marciani et al. 2001), and particularly in the intestines may influence the release of peptides such as CCK, GLP-1 and PYY(3-36) from the distal ileum and proximal colon (Cummings et al. 2007). Metabolites of gut fermentation, such as short-chain fatty acids (SCFA), have also been implemented in energy metabolism

(den Besten et al. 2013). These processes are dependent on the molecular properties of dietary fibre, the food matrix in which the fibre is delivered and its interactions within the digesta and GI environment. Both structural and functional properties and their potential influences on appetite are highlighted in **Figure 7**. Although dietary fibre could affect energy intake directly, there is also a possibility that dietary fibre could influence appetite without a corresponding direct effect on energy intake (as discussed in **section 2.6.1**).

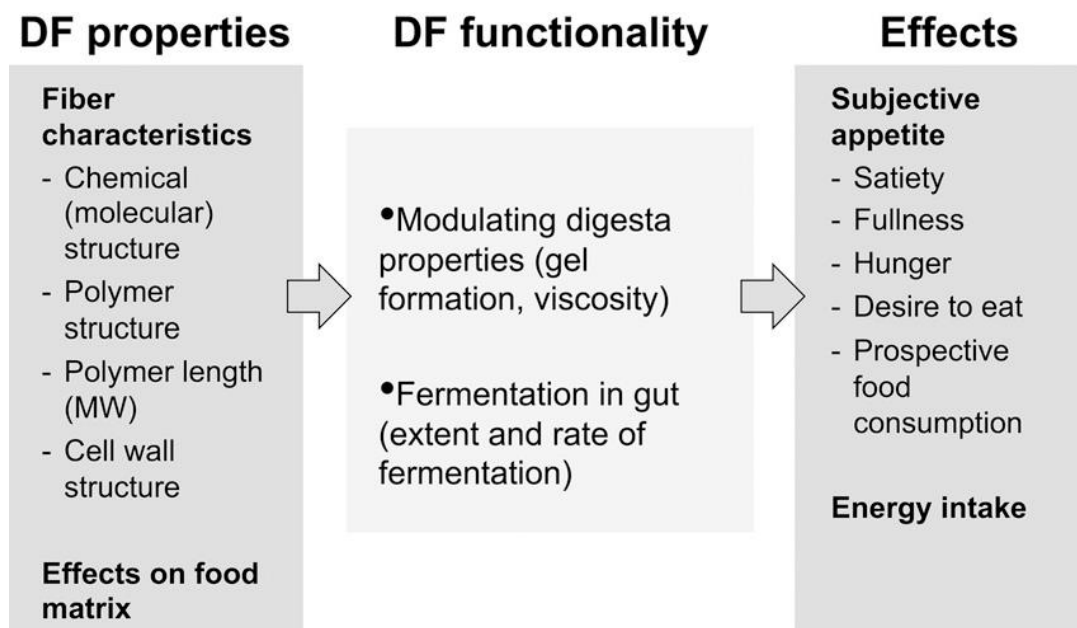


Figure 7. Structural and functional properties of dietary fibre and effects on eating behaviour. DF, dietary fibre; MW, molecular weight. Taken from Poutanen et al. (2017).

In both acute and long-term intervention studies, effects of dietary fibres on appetite and energy intake are variable and conflicting (Wanders et al. 2011; Clark and Slavin 2013). This is not unexpected because dietary fibres include a wide range of poly- and oligosaccharides with different compositions and structures that occur intact in foods, or are extracted as mixtures and/or extracted as individual compounds of variable molecular weight (MW) (Lupton et al. 2009; Raninen et al. 2011). Previous reviews have classified and compared fibres by their general class type and generic characteristics, with little focus on their physiochemical properties. The importance of dietary fibre characteristics has recently been discussed by Zaremba et al. (2017) and reviewed systematically by Poutanen and colleagues (2017), who prioritised such

physiochemical characteristics and evaluated their effects in appetite and energy intake in human intervention trials. The authors report that the majority of fibres included in the review (guar gum, pectin, psyllium, alginate, cereal β -glucan) that had high MW had a significant beneficial effect on appetite ratings in the acute setting. There was only one study however that used oat β -glucan, with a MW of 80 kDa and had no effect on satiety (Biörklund et al. 2008). It should be considered that the primary outcomes of the study by Biörklund et al. (2008) were not satiety or appetite, and the experimental conditions did not follow a typical acute appetite study design. Poutanen et al. (2017) only observed beneficial effects of high MW fibre on energy intake in 3 out of 10 studies in their review. This finding suggests that high MW as a characteristic itself did not consistently reduce appetite, as there is evidence of lower MW dextrin fibre (5 kDa) reducing hunger sensations (Guerin-Deremaux et al. 2011), yet an 80 kDa β -glucan reduced satiety (Biörklund et al. 2008). The review included only studies that detailed adequate information regarding physiochemical properties of dietary fibre, including name of dietary fibre and origin, along with at least one characteristic i.e., viscosity, gel formation, MW or fermentability, providing clarification of dietary fibre source and confirmation of properties postulated to influence appetite or energy intake. This is valuable for generating predictable structure-function relationships of dietary fibres rather than the experimental testing of dietary fibre mixes.

Whilst the systematic review by Poutanen and colleagues (2017) is the first to focus on the physiochemical characteristics of dietary fibres, there is currently no review of the literature specifically focussing on viscosity measurements of cereal β -glucan and their effects on appetite and eating, thus the following section will centre on this.

2.8.2 Influence of cereal β -glucan viscosity on appetite and energy intake

To the researcher's knowledge, there is currently no review of the literature which has identified the eating-inhibitory and/or satiety responses of studies that have characterized the physiochemical properties of cereal

β -glucan. Therefore, studies discussed in this section will have stated, as a minimum, the source of β -glucan, along with MW and/or viscosity and their effects on energy intake and/or subjective appetite.

In a crossover study by Beck and colleagues (2009a), breakfast meals containing a control (0 g), low (2.16 g), medium (3.82 g) and high (5.45 g) doses of oat β -glucan in extruded cereals were compared for their effects on satiety. Additionally, a cereal with an ethanolic extract of β -glucan with a dose of 5.65 g β -glucan was also given in a crossover fashion to 14 healthy overweight/obese subjects. A decrease in MW was offset by an increase in solubility to increase viscosity as the concentration of β -glucan increased in the cereals, with the MW of the test breakfasts reported to be 1.681×10^6 , 1.378×10^6 , 1.213×10^6 and 1.222×10^6 g/mol, respectively, which is in agreement with previous reports of the effects of extrusion on β -glucan (Izydorczyk et al. 2000; Zhang et al. 2011). As expected, the viscosities of the test breakfasts were dose-dependent, with the highest β -dose yielding the greatest peak viscosity, 84.8 mPa.s (range 5.8-84.8 mPa.s). There was an eating-inhibitory effect of ~96 kcal following the cereal containing 5.65 g β -glucan compared to the control cereal. In addition, subjective satiety increased across all doses of β -glucan, but not dose-responsively. The 5.45 g β -glucan dose significantly increased fullness compared to the other test breakfasts. In the same study, there was no effect of β -glucan on plasma ghrelin, however regression analysis reported a significant association between dose of β -glucan and plasma CCK ($r^2 = 0.97$, $p=0.002$) but was not apparent when the control condition was included. Acknowledging that seven is a small sample size, when subgroup analysis of females was performed, there was a relationship between control, low, medium and high β -glucan conditions ($p=0.036$, 0.032 , 0.006 , respectively). Beck and colleagues (2009b) extended their study to investigate PYY, reporting a significant correlation between PYY and fibre dose following regression analysis ($r^2=0.994$, $p=0.003$). The total levels of PYY increased linearly with increasing doses of 2.2-5.4 g β -glucan over the 4 hour inter-meal interval. The results from both studies by Beck and colleagues (2009a; Beck et al. 2009b) suggest that high MW β -glucan increased viscosity in a dose-response manner, however there was only an eating-inhibitory effect following the cereal with highest viscosity, which did not influence fullness ratings.

In studies evaluating the effects of oat-based breakfast cereals on subjective satiety, a 250 kcal serving of “old fashioned” oatmeal containing 2.6 g of β -glucan increased subjective satiety compared to an isocaloric oat-based ready-to-eat cereal (RTEC) containing 1.7 g of β -glucan. However, when a single serving of only 150 kcal of “old fashioned” oatmeal was compared to an isocaloric serving of the RTEC, the effect on satiety was less effective than that of the 250 kcal serving (Rebello et al. 2013; Rebello et al. 2014). Nevertheless, both serving sizes of instant oatmeal increased subjective satiety, while the 250 kcal serving of instant oatmeal also reduced energy intake. Unlike “old fashioned” oatmeal, instant oatmeal displayed a higher initial meal viscosity (after oral and initial gastric digestion) than the RTEC, 7397 mPa.s and 175 mPa.s, respectively (Rebello et al. 2014; Rebello et al. 2016a). It is possible that initial meal viscosity facilitates the induction of signalling via orosensory stimuli to influence the overall satiety response. Therefore, the crossover trials by Rebello et al. (2014) and Rebello et al. (2016a) support the results of other studies using MRI in which initial meal viscosity influenced satiety possibly via simultaneous oral, gastric and intestinal signals (Marciani et al. 2000; Hoad et al. 2004). There was a relatively large sample size in the studies by Rebello and colleagues, yet only their most recent study (Rebello et al. 2016a) measured physical food intake via *ad libitum* eating, which reported a significant decrease in ~85 kcal following the oatmeal cereal compared to control. The sugar content of the RTEC in each of the studies was also higher than that of the oatmeal used. Despite the kinetics of starch digestion and glucose release measured using *in vitro* mechanisms, with no differences found between cereals, it should be considered that differences in nutrient composition may have influenced study outcomes. Nevertheless, evidence from Rebello and colleagues are in agreement with Beck et al. (2009a), that cereals with greater viscosity have an inhibitory effect on eating. Yet despite this, high viscosity test meals have been shown to have no effect on satiety (Juvonen et al. 2009) or energy intakes (Peters et al. 2009; Clegg and Thondre 2014).

Following a standardized breakfast, energy intake and subjective appetite were investigated by Clegg and Thondre (2014) who compared two soups varying in β -glucan content and MW. A low MW soup containing 3.61 g barley β -glucan and high MW soup with 12.88 g barley β -glucan, with viscosities of 100 and 350 mPa.s, respectively, had no effect *ad libitum* eating at the test lunch 2 hours following soup ingestion, or

subsequent energy intake throughout the remainder of the study day. There was also no effect of either soup on subjective appetite ratings, suggesting that the physiochemical properties of barley β -glucan did not influence appetite ratings or actual energy intake in healthy normal weight males, despite one soup being considerably more viscous than the other. No effect on energy intake or subjective appetite were also reported following a β -glucan containing meal replacement bar (1.2 g barley β -glucan) in healthy overweight subjects, in spite of the viscosity of the β -glucan bar having a viscosity of more than double the control bar (841 mPa.s vs. 351 mPa.s) used in a study by Peters et al. (2009).

Other studies evaluated the effects of the viscosity generated by oat β -glucan on satiety by delivering the preload meal containing β -glucan in a beverage (Juvonen et al. 2009; Lyly et al. 2010). In a comparison among beverages (each approximately 167 kcal) containing 0 g, 5 g (2.5 g β -glucan) or 10 g (5 g β -glucan) fibre from oats, satiety was measured over a 3 hour inter-meal period. The fibre containing beverages increased satiety but not in a dose-dependent manner. Other conditions included in the study were a high viscosity beverage (167 kcal) containing 5 g β -glucan (661 mPa.s), a fibre containing beverage treated with β -glucanase enzyme to reduce viscosity (31.6 mPa.s), and a 0 g fibre beverage (1.5 mPa.s). The viscosities of the beverages were measured to identify the influence of viscosity on appetite, however MW or solubility of β -glucan were not measured or reported. The enzymatically treated beverage and the high viscosity beverage increased subjective satiety and reduced hunger compared to the 0 g fibre beverage, however there was no difference between the fibre-containing beverages on hunger ratings (Lyly et al. 2010). The beverage with enzymatically lowered viscosity produced greater satiety than the control beverage but less compared to the high viscosity beverage containing the same amount of β -glucan (2.5 g). The development of satiety and the effect of different food characteristics is therefore a very complex issue influenced by many factors.

Contrasting results were obtained when two isocaloric beverages (300 kcal) equal in volume, but differing in measured viscosity were compared (Juvonen et al. 2009). Each beverage contained 5.1 g β -glucan fibre from oat bran concentrate, with the viscosity of one test product reduced enzymatically using β -glucanase treatment. The study had a crossover design with 20 subjects, however no power analysis was conducted.

The low viscosity beverage (<250 mPa.s) produced significantly greater post-prandial CCK, GLP-1 and PYY responses compared to the high viscosity beverage (>3000 mPa.s). There was no difference in energy intake at the *ad libitum* test meal served 3 hours following test beverages, but the low viscosity beverage did produce an increase in subjective ratings of satiation. However, the authors reported that the high viscosity beverage delayed GE more than the low viscosity beverage. It is possible that the physiologic response may not change in proportion to viscosity, which is consistent with previous research (Dikeman 2006) or that beyond a certain degree of viscosity the response is insensitive.

In a study by Pentikäinen et al. (2014), subjects were served four different breakfast meals with β -glucan incorporated into either a solid and/or liquid matrix differing in β -glucan content. The test foods eaten for breakfast consisted of biscuits with a juice drink with 4 g β -glucan either added or not added to the biscuits and juice drink (55% orange juice and 45% water). Each type of biscuit was combined with each type of juice drink. The viscosity increased as the β -glucan content of the meal increased regardless of the food form; the viscosity of control biscuits and juice was very low (0.06 mPa.s), whilst the viscosities of the enriched biscuits and juice (7.7 mPa.s) and biscuits with enriched-juice (4.8 mPa.s) were higher and relatively similar to each other. The enriched biscuits and enriched juice (8 g β -glucan) was markedly more viscous (21.34 mPa.s) than the other combinations. While the addition of β -glucan increased perceptions of satiety compared to the control, the enriched juice and enriched biscuits combination produced the strongest effects on satiety. Moreover, the addition of oat bran was more effective in increasing satiety when added to the juice drink than when added to biscuits, which had the lower of the two viscosities with regards biscuit and juice enrichment. Thus, this study suggests that there is no dose-response relationship between viscosity and satiety, and corroborates the possibility that physiologic response may not change in proportion to viscosity. A critical drawback of the studies by Juvonen et al. (2009) and Pentikäinen et al. (2014), is that they failed to investigate the effect of viscous β -glucan on physical eating, i.e., they did not measure energy intake via *ad libitum* eating or food records. Despite the effects of high viscosity solids and drinks increasing satiety, it still remains unclear if there are inhibitory effects on eating using a viscous β -glucan-rich food matrix. It is also important to consider the practical implications of using a liquid matrix

to deliver β -glucan to the diet. In the two studies discussed, β -glucan was mixed into the liquid vehicle immediately before consumption to avoid excessive thickening of the product and reduced palatability, thus, this raises questions as to the practical significance of using liquid meals to deliver β -glucan (Juvonen et al. 2009; Lyly et al. 2010).

Most viscous dietary fibre solutions exhibit non-Newtonian, pseudoplastic flow, known also as shear-thinning, because their apparent viscosity values decrease when the shear rate increases. Due to this dependence on shear rate, measurements to characterize the flow behaviour of dietary fibre solutions should be made at a range of shear rates to obtain complete flow profiles (McKenna and Lyng 2013). For easier comparison with other findings, it is helpful to note the apparent viscosity at shear rates such as 10 or 50 s^{-1} , which have commonly been reported in the literature to reflect in-body conditions (Poutanen et al. 2017). However, there is no consensus on the actual shear rate most relevant to the stomach digestion phase because peristaltic movement produces mechanical forces that are difficult to simulate exactly as they occur *in vivo* (Verrijssen et al. 2014). The reporting of viscosity at other shear rates may also be justified by the hypothesized site of action. Reporting of shear rate was variable between studies reviewed in this section. Clegg and Thondre (2014) used shear rates ranging between 0.02-20 s^{-1} , whereas two studies failed to report shear rate (Peters et al. 2009; Rebello et al. 2016a), with one only stating rheometer rpm (Peters et al. 2009). Two studies did however measure viscosity at 50 s^{-1} (Juvonen et al. 2009; Lyly et al. 2010). Thus, there are very few studies present that report viscosity at a comparable shear rate, and conclusions cannot be drawn from the small amount of existing evidence. Additionally, there is also a lack of standardized protocols assessing eating-inhibitory effects, i.e., variable inter-meal intervals and *ad libitum* meal contents, that may give rise to inconsistencies reported in energy intake outcomes.

To summarize, only two studies have identified a modest eating-inhibitory effect (85 – 96 kcal reduction) following high viscosity test meals, with test meal viscosities of 84.8 mPa.s and 7220 mPa.s (Beck et al. 2009a; Rebello et al. 2016a). However, there is evidence of β -glucan test meals with viscosities within this range that have had no effect on eating (Peters et al. 2009; Juvonen et al. 2009) but do influence subjective

appetite ratings, however test food matrixes are considerably variable. Difficulties in identifying viscosity thresholds to modulate eating is not possible due to the lack of studies measuring energy intake and lack of consistency in rheological measurements with regards to shear rate.

2.9 The need to standardize *ad libitum* eating protocols

As discussed, acute effects of β -glucan on appetite and food intake are often inconclusive. As a result, the EFSA (2011) highlights that a direct cause and effect relationship has not yet been established between the consumption of dietary fibre and a sustained increase in satiety leading to a reduction in energy intake. Besides inadequate characterization of fibre chemistry, fibre source, matrix and dose of fibre administered in acute studies, poor standardization of the test meal paradigms is an important cause of the inconsistent results. Zaremba et al. (2017) recently reviewed the difficulties with regards to *ad libitum* eating protocols used in dietary fibre research, of which it was highlighted that statistical power, inter-meal intervals and test meal characteristics are important factors that are prone to vary considerably in the literature.

Not all studies that aim to investigate dietary fibre as a satiating agent measure *ad libitum* food intakes (Hlebowicz et al. 2008; Lyly et al. 2010; Pentikäinen et al. 2014; Rebello et al. 2014) relying instead on subjective appetite sensations, gut hormone responses and GE as indicators of actual eating. If *ad libitum* eating is assessed, energy intake is often not the primary outcome and, therefore, the study is not sufficiently powered to detect significant changes. Blundell and colleagues (2010) state that 20–25 subjects are generally sufficient to capture a 10% difference in mean AUC in appetite ratings between foods (not just specifically fibre), estimations that are based on subjective VAS scores. It is not surprising that studies that are powered to detect significant changes in subjective ratings may in fact not have a sample size large enough to detect changes in *ad libitum* energy intakes. Also, perceived appetite does not always parallel change in food intake (Blundell et al. 2010; Almiron-Roig et al. 2013), giving rise to conflicting findings across acute intervention studies.

2.9.1 The inter-meal interval

Inconsistent findings in fibre preload studies may be also affected by poorly controlled timings of the *ad libitum* meal following an initial fibre preload. Inter-meal intervals have been reported to range considerably between studies, with *ad libitum* test meals administered from 1 hour (Samra et al. 2007) up to 5 hours (Rebello et al. 2016a) after fibre preloads. It is well established that gastric distention and intestinal (hormonal) signals synergistically mediate early fullness and satiation and that GE modulates both of these processes (Langhans and Geary 2010; Steinert et al. 2012). High viscous fibres slow GE leading to increased stomach distention and also delay small bowel transit, resulting in a prolonged presence of chyme in the small intestine (Bergmann et al. 1992; Howarth et al. 2001; Slavin 2005). The latter slows nutrient absorption and may also beneficially modulate the secretion of satiation hormones (Beck et al. 2009a; Ho et al. 2015). For example, it has been suggested that increases in GI satiation hormones and the slowing of GE have a key role in the satiating effect of viscous fibres such as oat β -glucan (Beck et al. 2009a; Beck et al. 2009b; Juvonen et al. 2009). A previous systematic review by Wanders et al. (2011) documents that viscous fibres (e.g., pectins and β -glucans) reduce acute energy intake more than less viscous fibres (e.g. arabinoxylan) by 69% and 30%, respectively. Therefore, for viscous fibres, it may be important to administer the *ad libitum* meal shortly after the fibre preload (30–120 min) in which GE is still ongoing. This is significant because as the stomach continues to empty, it will have a gradually decreasing role in eating (Janssen et al. 2011). Because fibre viscosity depends on MW and fibre solubility (Ahmad et al. 2012), these characteristics will also influence satiating mechanisms. Moreover, solid food empties from the stomach more slowly than both low and high nutrient dense liquid food; (Marciani et al. 2000; Achour et al. 2001) thus, the food matrix of which the fibre is delivered also has a pivotal role. In contrast, inter-meal intervals of 4–5 hours or more may be more suitable for fermentable fibres that are suggested to influence appetite processes via the production of SCFA produced as a result of colonic microbial fermentation and the subsequent release of GLP-1 and PYY (Noack et al. 2013; Chambers et al. 2015). Some research also suggests ‘second meal effects’, i.e., fermentable fibre may modulate not only the first

subsequent meal after consumption but also later meals on the same or even subsequent day (Isaksson et al. 2008; Ibrügger et al. 2014). Clark and Slavin (2013) systematically reviewed the effect of fibre on appetite perceptions and food intake and found that the greatest reduction in food intake is seen when *ad libitum* meals were given 4–5 hours post fibre consumption in the acute appetite study setting. They also report that the most frequent inter-meal interval period for administering the *ad libitum* meal was between 3 and 3.9 hours. It is important to note that this observation did not differentiate between different fibre characteristics but included studies with both viscous and fermentable fibres, an avenue that warrants further investigation.

2.9.2 Test meal characteristics

There is also little consensus as to the form, content and nutritional composition of the *ad libitum* test meal in fibre-based studies. Appropriate measurement of satiation needs to take into consideration the properties of food and environmental factors that are involved in meal termination, including sensory factors. With reference to the satiety cascade (Blundell et al. 2010) it is evident that sensory and hedonic factors have an important role in meal termination. It is well documented that the palatability of a food has a positive effect on the amount eaten in both male and female obese and non-obese subjects (Sørensen et al. 2003). However, despite the importance of ‘liking’ or ‘disliking’ particular ingredients that make up the test meals, palatability is often not reported. Additionally, it is also important to consider boredom with taste as a factor inhibiting food intake, a concept referred to as ‘sensory specific satiation’ (Hetherington 1996). A wide range of different test meals to measure *ad libitum* eating, including isocaloric portions of ham sandwiches (Steinert et al. 2010), macaroni and beef (Geary et al. 1992), pizza (Samra et al. 2007), or self-selection buffet-style lunches (Luscombe-Marsh et al. 2013) are reported in the literature. When subjects are offered a wider range of foods they will consume more than in situations where choice is limited (Mok 2010; Hetherington et al. 2006). To what extent different types of *ad libitum* test meals influence the satiating capacity of a given fibre preload has not been investigated to the researcher’s knowledge and warrants

further investigation. In order to better compare across different fibre interventions, it would be useful to use an accepted standard meal, which does not vary in energy content, taste, texture or nutritional composition, as altering these parameters may cause small shifts in transient satiety.

Inconsistencies in food intake when investigating dietary fibre as an appetite suppressant in the acute study setting may continue to give rise to conflicting findings so long as non-standardized eating protocols are adopted. The goal of any appetite suppressing food is to reduce energy intake; yet although subjective appetite feelings may imply satiation, this does not always translate into eating less. The following test meal parameters should be considered more frequently besides detailed characterization of fibre chemistry, fibre source, matrix and dose of fibre administered.

The primary outcome of fibre studies should be *ad libitum* food intake, and this should be powered accordingly with an appropriate sample size to detect changes in energy consumed. The inter-meal interval should be determined in relation to the type of fibre under investigation. Tailoring the inter-meal interval around the physiological mechanisms (GE, hormonal secretion and colonic fermentation) by which each type of fibre functions is critical. An inter-meal period of more than 4 hours may be a more realistic timeframe for fermentable fibres in the preload experimental setting. In contrast, for fibres that modulate eating mainly via their viscous nature, an inter-meal interval of 30–120 minutes may be more appropriate. A standardized *ad libitum* test meal would allow for appropriate comparisons to be made across studies that investigate the same fibre types, and may ultimately help identify fibres with the greatest satiating effect. Because hypothetically acute satiating effects seen in the short term should translate into longer-term effects, that is, deficits in energy consumed lead to weight reduction, standardization of the above parameters should identify efficient satiating fibres to be investigated in the long term. Such approach may strengthen the evidence for the role of fibre in weight management.

In summary, part of the dilemma in our ability to derive clear answers to the possible role of dietary fibres in appetite control may be, from a methodological perspective, the lack of standardization of *ad libitum* eating protocols currently in place. Although this is by no means a novel revelation, it must be reiterated

that until methodologies for measuring satiation are made more universally robust, the exact satiating role of dietary fibre upon appetite may not be forthcoming.

2.10 Longer-term soluble fibre consumption

The degree of weight loss induced by dietary fibre is believed to be greater in those who are obese, corroborated by a meta-analysis by Howarth and colleagues (2001) which reported that fibre consumption of >14 g per day reduced energy intake by 10% and reduced weight by 1.9 kg over a ~4 month period. Soluble dietary fibre supplementation has been shown to prove effective as an adjunct to hypocaloric diets in individuals who were overweight. In a placebo-controlled intervention by Birketvedt et al. (2000), 53 moderately overweight females (BMI >27.5 kg/m²) followed a calorie reduced diet with and without fibre supplementation of 4 g glucomannan daily for 24 weeks. After treatment the degree of weight loss was larger in the fibre supplemented group compared to the placebo group, with 8 kg and 5.8 kg reductions in body weight reported, respectively. Similar effects have also been seen in a follow up investigation by Birketvedt and colleagues (2005). A reduction of 3.8 kg in weight was reported in healthy overweight females who consumed 1.24 g per day of glucomannan daily for five weeks in conjunction with dietary and exercise counselling. PolyGlycopleX® (PGX) is a synthetic, highly viscous soluble fibre derived from glucomannan, which possesses the highest water holding capacity of currently known fibres. The use of PGX with general changes in diet and physical activity over a 14 week period has been shown to play a beneficial role in modifying cardiometabolic risk factors in overweight and obese subjects. Lyon and Reichert (2010) reported a 5.79 kg reduction in weight and 12 cm in WC measurements following a 5 g PGX fibre preload 2-3 times daily before meals. In addition, fasting plasma low-density lipoprotein cholesterol (LDL) reduced by 25.5%, fasting glucose by 6.9% and insulin by 27.3%. The administration of this fibre offers promising effects in terms of improving health status in overweight individuals, however it must be noted that no placebo-control was used during the intervention period, thus it cannot be said the aforementioned effects were down to PGX administration alone. Administering soluble fibre in conjunction

with lifestyle changes makes determining the efficacy of soluble fibre supplementation difficult; it is important to investigate the effects of soluble fibre consumption without altering lifestyle in order to gauge its effectiveness in terms of weight control alone. Moreover, few studies fail to distinguish between the different types of fibre, thus it is unclear if a specific fibre, i.e., β -glucan, is more or less effective in reducing weight. Significant decreases in mean body weight (-1.03 kg), BMI (-0.38) and WC (-1.63cm) were reported in a group of overweight volunteers in an investigation conducted by Liatis et al. (2009). The T2DM patients consumed bread daily enriched with 3 g β -glucan for a period of three weeks and saw significant decreases in anthropometric parameters when compared to baseline measurements. On the other hand, Robitaille et al. (2005) saw no significant changes in anthropometric measurements following a four week intervention period in overweight women who consumed oat bran-enriched muffins, delivering 2.32 g β -glucan per day. It can be suggested that the daily dose of β -glucan was perhaps not large enough to elicit a weight loss effect in the volunteers, however larger doses of β -glucan have yielded similar non-significant results in terms of body weight and BMI even when consumed for a longer duration of time. Queenan et al. (2007) report no effect of oat β -glucan concentrate (6 g β -glucan imbibed at breakfast and dinner) consumed for six weeks on body weight compared to a placebo group who received dextrose monohydrate as a control. There was also no effect on body weight following 4 g β -glucan-rich soup (400 g serving of 270 kcal) per day consumed for five weeks by overweight individuals (Biöklund et al. 2008). In a six week parallel, randomised, controlled, single-blind trial, oat based cereal bars containing 3.2 g β -glucan were compared to RTEC (oat flakes and puffed rice and wheat bars delivering 1.5 g and 0 g per day β -glucan) eaten daily by overweight subjects. All groups lost weight (-1.0 kg) however there were no statistical differences between groups, suggesting that neither dose of β -glucan aided weight loss (Charlton et al. 2012). In the same study, there were no significant interaction effects between groups for energy and macronutrient intakes, although all groups reported within-group reductions in energy intakes and in percentage of energy from total fat and saturated fat (SFA). Since this was seen in all groups, it can be suggested that this may have been as a result of the low-fat healthy eating advice subjects received at the start of the study, and not from a satiating perspective from oat β -glucan consumed daily.

Further research is warranted with regards to repeated daily consumption of β -glucan-rich foods, particularly when it is pertinent to point out that body weight reduction was not the primary outcome in most studies but instead these interventions were powered to detect changes in cholesterol. Little is known with regards to the impact on energy intake when diets rich in β -glucan are consumed, particularly with regards to the displacements of macro- and micronutrient profiles.

2.11 Blood pressure

Although evidence on increasing fruit and vegetable consumption is included in guidance to reduce blood pressure (BP), advice on fibre consumption is not (Evans et al. 2015). Two reviews have previously highlighted an inverse relationship between fibre consumption and BP (Streppel et al. 2005; Whelton et al. 2005), however, both reviews did not describe effects by fibre type. Efficacy of diets containing β -glucan consumed daily in subjects with normo- and hypertension is inconsistent, with decreases in BP accompanied by weight-loss.

Davy and colleagues (2002) randomly assigned 36 study participants with elevated BP to consume 14 g per day of dietary fibre in the form of oat or wheat cereals for 12 weeks. They found that casual and ambulatory BP did not significantly change over the 12 week intervention period. Overweight and obese non-medicated subjects who had high-normal BP were randomly assigned to receive one of two sets of food products for a 12 week treatment period in an investigation by Maki et al. (2007). Subjects followed either a control diet, which contained no oat β -glucan, or a β -glucan-rich diet that delivered 7.7 g β -glucan daily. Following the 12 week intervention period, neither systolic blood pressure (SBP) nor diastolic blood pressure (DBP) showed statistically significant differences between treatment groups at any time point in the overall study sample. However, following BMI subgroup analysis, using the median value as a cut-point, subjects in the high-BMI group ($> 31.5 \text{ kg/m}^2$), both SBP and DBP showed significant differences between the β -glucan and control treatments. Subjects in the high-BMI β -glucan group showed mean baseline to end-of-treatment changes in SBP and DBP of 5.6 and 2.1 mmHg, respectively, whereas high-BMI control subjects showed

increases of 2.7 and 1.9 mmHg. It is unclear whether these changes in BP were apparent as a result of weight change, as body weight was not measured in the study. Dietary changes other than soluble fibre intake could, however, explain some of the BP-lowering effect. Reductions in sodium and calcium intake and an increase in potassium intake was observed in patients assigned to the β -glucan group.

A weight-loss diet containing oats was associated with favourable decreases in SBP compared with a control diet without oats in a study by Saltzman et al. (2001). The oat diet was designed to deliver 45 g oatmeal per 1000 kcal per day. A mean decrease in 6 mmHg (± 7 mmHg) was recorded in overweight subjects who followed the oat diet, which was accompanied by a loss in 5.5% body weight, of which 2.7% was body fat, over an eight week period. Despite the subjects being normotensive, a decrease in SBP was reported. The study failed to detail the exact changes in body weight over the intervention period, and additionally the caloric deficit of 1000 kcal per day followed during the study can be considered excessive, considering that the subjects had a baseline BMI of around 26 kg/m². Nevertheless, the study highlights the benefits of incorporating 45 g oatmeal per day in conjunction with a hypocaloric diet in overweight subjects. Modest changes in weight have also been reported alongside decreases in BP in subjects consuming oat β -glucan daily over a minimal period of six-weeks (Davy et al. 2002; He et al. 2004; Charlton et al. 2012). The efficacy of water-soluble fibre intake from high-fibre oat bran snacks (15.9 g fibre, 7.3 g β -glucan daily) on BP in a free-living population of high-normotensive healthy subjects was compared to low-fibre dietary snacks (2.7 g fibre, 0 g β -glucan). Although the difference in BP reduction between the high and low fibre intervention groups was not statistically significant, the within group results suggest that a high fibre snacks may reduce BP. Both SBP and DBP were significantly reduced in the study participants who consumed high fibre dietary snacks. In contrast, BP was not significantly reduced in those who consumed the low fibre diet. Modest reductions of mean SBP and DBP within the high-fibre consuming group, 3.4 mmHg and 2.1 mmHg, respectively, was not accompanied by weight change, which suggests that changes in BP were independent of weight loss. The BP attenuating mechanism of β -glucan is unclear. Keenan et al. (2002) found BP reductions of 7.5 mmHg SBP and 5.5 mmHg DBP in hyperinsulinaemic men and women with hypertension who consumed oat cereal containing 5.5 g per day of β -glucan. A trend toward

a lower insulin response to an oral glucose load was observed in the oat cereal group, whereas there was no change in the control group that consumed a low-fibre wheat cereal. Insulin sensitivity, measured with minimal model evaluation of glucose and insulin responses to an IV glucose load, was unchanged in either group. This suggests that the ability of viscous soluble fibres to lower BP may not be dependent on enhancement of peripheral insulin sensitivity. A recent meta-analysis by Evans et al. (2015) reports that diets rich in β -glucan reduce SBP by 2.9 mmHg and DBP by 1.5 mmHg for a median difference in β -glucans of >4 g. Studies included were either parallel or crossover in nature of at least 6 week in duration, of which only 5 studies were suitable for SBP analysis and 4 for DBP, highlighting the lack of robust studies for β -glucan and BP.

These studies indicate that increased intake of β -glucan can reduce BP and suggest that dietary sources naturally rich in cereal β -glucan and β -glucan-supplemented foods as part of a healthy diet could be recommended to hypertensive patients. A decrease in BP may not only depend on the dose of β -glucan but might also be linked to changes in body weight, total energy and dietary intake, which in turn might be only partly dependent on β -glucan consumption. Mechanisms behind the BP-lowering effect of dietary fibre are, however, still unclear, despite the positive findings reported from the meta-analysis by Evans and colleagues (2015). More human intervention studies are needed to confirm the BP-lowering effect of β -glucans within overweight and obese populations and to define its optimal use in different settings, with careful measurement of dietary intakes and body composition to assess their impact on BP effects.

This chapter has demonstrated that there is conflicting evidence regarding the effectiveness of β -glucan-enriched meals and/or diets to positively influence energy intakes in both the short and medium term. With roles in eating, GE and glycaemic control, GLP-1 is biomarker of interest in appetite research, particularly when evidence of GLP-1 responses following β -glucan consumption is limited. More evidence is required to identify the true satiating nature of β -glucan in the acute setting, in addition to gaining more insight into what effect, if any, β -glucan consumption has on daily energy intakes and if these effects may impact on body composition and markers of metabolic health. There is lack of evidence that investigate the effects of

oat β -glucan on eating that have focussed on using energy intake (kcal) as the primary study outcome. As daily energy intake can be largely variable (Rolls et al. 2002; Muller et al. 2015), it is important for studies that aim to explore possible eating-inhibitory effects following β -glucan consumption to be adequately powered to detect changes in energy intake. Detecting changes in physical eating is a more nutritionally relevant parameter and would present more clinical value than detecting changes in perceived ratings of satiety alone. Therefore, both studies in this thesis were designed with energy intake as the primary outcome measure. Additionally, in appetite and energy intake studies, it is critical that test meals and/or snacks are appropriately matched to suitable controls. Discrepancies in macronutrients have been previously reported between test and control meals. For example, Rebello et al. (2013) report a carbohydrate content of 45.09 g in Quaker Oatmeal vs. 49.97g carbohydrate in Honey Nut Cheerios used in two “matched” test meals. In another publication by Rebello and colleagues (2014), there is a discrepancy of 3 g carbohydrate between oatmeal and Honey Nut Cheerios (27 g vs. 30 g). Changes reported in appetite and satiety by Rebello and colleagues cannot be attributed to by oat β -glucan content as meals were not adequately matched in terms of their nutritional profile. The current work presented in this thesis used appropriate controls and therefore increased the scientific robustness of the research performed.

The two studies in this PhD thesis aimed to challenge the hypothesis that individuals who consume oat β -glucan, whether acutely or over a longer period of time, will reduce their energy intake. Acute satiety and other long-term benefits were investigated, along with other appetite parameters, such as GI hormones, in order to inform mechanisms of action.

2.12 Aims and Objectives

The aim of this PhD research was to investigate whether oat β -glucan had any impact on energy intakes and appetite markers in the short term. Furthermore, regular consumption of oat β -glucan within a novel food product was also explored to assess energy intakes and risk factors associated with the development

of the MetS in an overweight population. Ultimately, this PhD research will contribute to the body of scientific evidence used to examine the possibility of a health claim related to oat β -glucan, energy intakes and weight management.

2.12.1 Research Questions

1. Does incorporating oat β -glucan into the diet of healthy individuals reduce energy intake in the short and medium term?
2. Does oat β -glucan elicit effects on markers of satiety and satiation, such as subjective appetite ratings and GI hormonal responses?
3. Does daily consumption of a novel β -glucan-enriched oatcake influence energy intakes of overweight/obese subjects?
4. Does consuming oat β -glucan daily influence the metabolic profiles of those who are overweight and/or obese?

To test these hypotheses and to achieve these overall aims, a preliminary short-term intervention, *Effects of oat β -glucan consumption at breakfast on ad libitum eating, appetite, glycaemia, insulinaemia and GLP-1 concentrations in healthy subjects* (Study A), was carried out over one day to investigate the impact of a single meal containing oat β -glucan on appetite parameters and subsequent food intake. Furthermore, a medium-term intervention, *Effects of a six week intervention with novel β -glucan-enriched oatcake snacks on daily energy intakes, body composition and markers of metabolic health in overweight and obese individuals: a pilot study* (Study B), was carried out over a period of six weeks to investigate whether there was a cumulative effect of incorporating oat β -glucan into the diet via daily consumption of β -glucan-enriched oatcakes on energy intakes, body composition and markers associated with the development of the MetS.

Chapter 3

3.0 Effects of oat β -glucan consumption at breakfast on *ad libitum* eating, appetite, glycaemia, insulinaemia and GLP-1 concentrations in healthy subjects (Study A): Methodological Considerations

There are already many food products with claims regarding health effects beyond the simple provision of nutrients. One important basis for claims is the increasing number of reports of the effects of dietary components on body functions.

The Process for the Assessment of Scientific Support for Claims on Foods (PASSCLAIM) aim to evaluate existing schemes which assess scientific substantiation, produce a generic tool for assessing the scientific support for health claims for foods, and establish criteria for markers which can be used to explore the links between diet and health.

Since the PASSCLAIM criteria outline outcome measures of weight control together with markers related to appetite, the PASSCLAIM criteria were used, in part, as framework to aid designing both studies in this thesis, with the goal of gathering significant evidence related to oat β -glucan. With regards to β -glucan there are a number of steps required before reaching the goal of health claims related to weight management (Ricardi et al. 2005). It is important to;

- clarify that the product under investigation contains functional components
- investigate proposed mechanisms such as acute satiety and related hormonal responses
- review the functional components within a dietary regimen in humans

As mentioned in **Chapter 2 (section 2.8.2 and 2.9.1)**, the ability of β -glucan to form a viscous bolus in the GI tract is the main mechanism of action by which this soluble fibre works. There is a positive non-linear relationship between MW and viscosity, with the MW of β -glucan being subject to oat cultivar variety, growing conditions, processing and storage (Aman et al. 2004; Ajithkumar et al. 2005). The MW of purified

oat β -glucan is in the range of 50 – 3000 kDa (Ajithkumar et al. 2005) but is decreased by food preparation such as bread-making or further extrusion that impacts bioactivity of cereal β -glucan (Tosh et al. 2008; Tosh et al. 2010; Wang and Ellis 2014). Therefore, the first aim of any research investigating β -glucan must be to use a β -glucan source integrated into a product/food matrix which will be of sufficient concentration, MW and solubility to yield high viscosity in the GI tract following consumption (Regand et al. 2011). The first study (A) in this thesis relates to acute energy intake, satiation and mechanisms involving incretin related hormonal responses; glucose, insulin and total GLP-1. Previous research has incorporated β -glucan into muffins (Willis et al. 2009), breads (Vitaglione et al. 2009; Finocchiaro et al. 2012), biscuits (Pentikäinen et al. 2014) and soups (Cugnet-Anceau et al. 2010; Clegg and Thondre 2014), which are arguably not easily produced or available freely to purchase. Thus, emphasis was put on designing a test breakfast which would be relatively inexpensive to replicate and practical to make by the general public using a commercially available oat bran powder.

Ultimately, research related to food should have commercial application and thus product development is important for mechanistic and organoleptic factors. Development of a novel β -glucan-enriched oatcake was undertaken by Nairn's Oatcakes Ltd., Edinburgh, and used as a means of delivering oat β -glucan to the diet over a period of 6 weeks in healthy overweight and obese adults. Because hypothetically acute satiating effects seen in the short term should translate into longer term effects, i.e., deficits in energy consumed lead to weight reduction, it is important to investigate the effects of daily consumption of β -glucan-enriched products in order to identify whether or not changes in energy intakes and body weight will be seen under these conditions. This was the focus of the second study (B) in this thesis.

Measurement of energy intake with subjective satiety together is critical to link any satiety measures with positive outcomes in both the short and long term. The relative usefulness of energy intake and measurements of satiety are thus reviewed in this chapter, in addition to the benefits and drawbacks of using such methodologies. Applying this together in appropriate experimental designs is the final methodological consideration and is discussed at the end of this chapter.

3.1 Measuring Appetite Parameters

Appetite is a subjective construct and is not open to direct measurement (Mattes et al. 2005). Appetite is often divided into three components: hunger, satiety and satiation. Heavily influenced by metabolic, sensory and cognitive facets, hunger describes the sensations that promote food consumption (Weingarten 1985). Satiety is the process that leads to feelings of fullness that persists after eating, with the potential to inhibit further food intake until hunger returns. Satiation on the other hand is referred to as the process that leads to the termination of eating and, thus, controls meal size (Benelam 2009). From a research perspective, focus has been on increasing the satiating power of diets so that individuals feel full with fewer calories (Astrup 2005), therefore investigating foods that improve satiety and satiation are important in energy balance and obesity research. In order to achieve good practice in carrying out appetite research, as part of the expert group International Life Sciences Institute (ILSI), Blundell et al. (2010) report key considerations that should be addressed in any such appetite research that aims to quantify hunger, satiety and satiation. Food intake data, subjective measurements and biochemical measures of hormones are three assessment methods most commonly used. It is important to mention that other methods such as the microstructure of eating (counting the number of chews and rate of eating) and salivation have been used to measure appetite, however they are not widely accepted as valid indices of appetite (Spiegel 2000; Yeomans 2000).

Generally, in acute appetite studies, short-term prandial effects of an intervention are assessed using a single day preload–test meal paradigm (Blundell et al. 2010). Driven by the ease of completion and the relatively low cost of running, preload studies have emerged as the cornerstone for appetite research, and consequently are used often by food companies who are seeking validation of satiety claims for foods and nutrients. These studies measure the effects of a specific nutrient, food or meal ‘preload’ on postprandial appetite-related ratings such as hunger and fullness, measured via VAS ratings and quantify *ad libitum* food intake from one or more subsequent meals. In many preload studies, food intake for the remainder of the day is often self-reported by subjects. If the food or nutrient preload alters subjective appetite sensations, it

can be hypothesized that this will, as a consequence, alter eating behaviour and that such change can be quantified by measuring *ad libitum* food intake at the outcome test meal. A number of adaptations to the preload test meal paradigm have been made. This includes manipulation of the test meal to include potential satiating agents, which arguably will be more externally valid in that they simulate real-life feeding situations (Blundell et al. 2010).

3.1.1 Subjective measures of satiety

Unlike animal research, humans can provide unique insights into eating behaviour because of their capacity for reflection. Visual analogue scales are the most common questionnaires used to assess subjective feelings of appetite. The VAS questionnaire is composed of lines 100 mm in length, anchored by word descriptors at each end describing the extremes of a characteristic or attitude that range across a continuum of values (Livingston et al. 2000). Hunger, fullness, satisfaction, desire to eat and prospective food consumption are the most common parameters typically monitored. Individuals are asked to make a point on the 100 mm line which best represents how they feel at a particular time. Visual analogue scales are completed before and after consumption of a test product and then again at regular time intervals, administered every 15-30 minutes up to 1 hour, usually over a duration of 3-5 hours (Blundell et al. 2010). **Table 3** highlights the recommended primary scales for assessment of self-reported appetite in healthy adults.

Table 3. Recommended primary scales for subjective appetite ratings. Taken from Blundell et al. (2010)

Scale	Question	Anchors	
		<i>Low</i>	<i>High</i>
Hunger	How hungry are you?	Not at all	Extremely As hungry as I have ever felt
Fullness	How full are you?	Not at all	Extremely As full as I have ever felt
Satiety	How satiated are you?	Not at all	Extremely
Desire	How strong is your desire to eat?	Very weak Extremely low	Very strong Extremely high
Prospective consumption (quantity)	How much do you think you could (or would want to) eat right now?	Nothing at all	A very large amount

The degree to which individuals understand the subtle differences between these questions is uncertain, in addition to the ability of these questions to accurately assess appetite and subsequent energy intake. In a tightly controlled laboratory setting by Flint et al. (2000), the correlation between pre-lunch ratings of appetite and energy intakes was reported to be significant but only on a weak to moderate scale. This is in accordance to the many studies that have evaluated short and long-term relationships between eating motivation and energy intake, with most studies identifying a statistically significant relationship (Hulshof et al. 1993; Porrini et al. 1995; Parker et al. 2004). It is important to highlight that there was a lack of consistency between these studies, with some studies utilizing different eating motivation parameters, such as satiety quotient and pre and post meal ratings. In addition, further differences in methodological design were apparent, such as small sample sizes, populations and use of different appetite related questions. As a result, findings are variable, power is often limited, and the effect in relation to other subject characteristics, such as gender, body weight and age, have been lacking and at times contradictory (Doucet et al. 2003). Utilization of statistical analysis to identify differences between measures is also a major drawback.

Although commonly done, analysis of subjective appetite parameters at multiple individual time points (e.g. 30, 60, 90 minutes etc.) is not recommended (ILSI expert group; Blundell et al. 2010). Analyzing satiety data on a single time point basis does not take into account that satiety responses are a function of multiple time points, and these individual time points are not physiologically or statistically independent. Despite inconsistencies in the literature between VAS ratings and energy intakes, the use of VAS are currently the most established tools to assess subjective appetite and are commonplace in appetite research. According to Blundell et al. (2010), VAS have generally good repeat-reliability with regards to group mean data and comparisons of specific foods. In a reproducibility, power and validity orientated study conducted by Flint et al. (2000), it was suggested that 8-35 subjects would be required to identify a 10% change (10 mm) difference in 4½ hour mean appetite ratings between two foods, which they consider to be a “reasonable and realistic difference”.

In order to explore a potentially more intuitive and precise option to VAS scales, Sadoul et al. (2012) explored a method based on pictures. Individuals were asked to indicate how many portions of each pictured product they would like to eat, which was then later compared to VAS ratings scored at the same time. It was reported that picture scores were more sensitive than VAS in differentiating intervention effects on satiety, however despite this difference, it is important to consider that this may have been as a result of simultaneous completion of both tasks and may have artificially raised the correlations between them. Area under the curve calculations were also based on 2-4 timepoints, which may have led to an increase in experimental-wide error. The results from the picture based method is promising, but validation would be required for pictures to become a standard and accepted alternative to VAS.

When large scale trials are investigating the effects of interventions on appetite, it is critical to efficiently monitor large amounts of human data (Gibbons et al. 2011). Electronic appetite rating systems (EARS) have been developed to aid ease of completion, particularly since administering multiple paper VAS can be laboursome to both researcher and participant. Time and data are monitored with EARS, which assists

in ensuring integrity of the data collected. Yet despite tabulation of data from EARS being much easier compared to paper and pen VAS, it is important to point out that there are differences in the actual length of the line, and as a result of this individuals can be less likely to mark on the extremes of the lines. For this reason, EARS must not be used interchangeably with pen and paper VAS (Stubbs et al. 2001).

Category scales differ from VAS in that rather than using one continuous line, a category scale will divide the line into distinct categories. The major drawback with this method is that this means of scale is subject to psychometric limitations. For example, a hunger rating of eight should not be interpreted to mean that it is twice as strong as a hunger rating of four (Mattes et al. 2005).

3.2 Energy intakes

The primary long-term goal of appetite research is weight control, and dietary intake is directly linked to changes in energy balance (Hall et al. 2012). While biochemical markers are used in attempt to determine physiological mechanisms, and subjective scales are used to quantify perceived feelings and intent to consume food, ultimately appetite is investigated to help individuals consume more or less in relation to their state of energy balance. With this in mind, energy intakes should be quantified in any given appetite study, regardless of the dietary intervention implemented.

Dietary intake refers to the daily eating patterns of an individual, including specific foods and calories consumed and relative quantities. Just because satiety may be elevated at a single time-point does not necessarily mean that there will be a reduction in total energy intake – intake can simply increase during a subsequent meal.

Knowing how long the effects from an individual food item will last for, or if there will be potential compensatory eating behaviours during subsequent times in the day, is difficult to determine. It is well established that reducing energy intake over time will result in weight decrease (Joosen et al. 2005) thus

measuring energy intakes not only serves as a rational proxy for weight loss but is a useful tool for quantifying compliance and energy expenditure.

3.2.1 *Ad libitum*

Satiation is evaluated by the measurement of *ad libitum* (at one's pleasure) food consumption of particular experimental foods (kcal or kJ) under standardized conditions (Blundell et al. 2010). As mentioned, measurement of satiation is important as it is the process that brings a meal to an end and therefore determines the size of an eating occasion. In real life most eating occasions and/or snacking are terminated by environmental cues, such as portion size. It is normal for us to finish a meal and leave a 'clean plate', a behaviour that is installed in us from childhood (Sheen et al. 2018). An example of such behaviour was shown in an observation study by de Graaf et al. (2005). In a field study of soldiers, who consumed in total around 5700 main meals, it was observed that soldiers ate 100 % of their meal 80-90 % of the time, even when their meal was not particularly liked.

Ad libitum consumption of foods varies by a large extent. Weenen et al. (2005) reported that 70-80 g savoury cheese biscuits were required to be eaten for satiation to be reached in a group of normal weight and overweight women, whereas when pears in syrup were the *ad libitum* option more than 350 g were eaten. This was not as a result of the pears being liked more than cheese biscuits, both were equally liked and accepted. Many studies have highlighted that palatability is a strong determinant of *ad libitum* energy intake, including studies that are experimental (de Graaf et al. 1999) and real-life (de Graaf et al. 2005) in nature. Furthermore, energy density is an important factor. If we consider the energy density of the aforementioned cheese biscuits to be 540 kcal per 100 g and the pears to be around 65 kcal per 100 g, we can see an eight-fold difference in energy density between these two *ad libitum* meals. Comparing intakes from these meals would be unrealistic and would not be likely to capture the satiating nature of a given food. Therefore, when designing an appetite study it is critical that the *ad libitum* meal is carefully planned.

There is little consensus as to the form, content and nutritional composition of the *ad libitum* test meal across appetite literature. There are no standardized meals or validated protocols that are universally accepted. As mentioned, appropriate measurement of satiation needs to take into consideration the properties of food and environmental factors that are involved in meal termination, particularly sensory factors. With reference to the satiety cascade (Blundell et al. 1987), it is evident that sensory and hedonic factors play an important role in meal termination. It is well documented that the palatability of a food has a positive effect on the amount eaten in both male and female obese and non-obese subjects (Sørensen et al. 2003). However, despite the importance of ‘liking’ or ‘disliking’ particular ingredients that make up meals, thus, influencing food consumption, palatability is often not reported in the literature with regards to *ad libitum* test meals. Factors that should be addressed when planning *ad libitum* test meals has previously been discussed in **section 2.9**. Particular focus should be on powering studies with energy intake as the primary outcome and determination of an appropriate inter-meal interval.

3.2.2 Diet diary

The appropriate tool for dietary assessment depends on the purpose for which it is needed. Many different methods have been developed for the purpose of assessing dietary intake. These range from detailed individual weighed records collected over a period of up to seven days, to food frequency questionnaires, household survey methods and simple food lists; each with merits and practical difficulties (Shim et al. 2014). The most rigorous method is the weighed intake, which involves an individual or researcher weighing each and every item of food and drink prior to consumption. A 7-day weighed record is considered as the ‘gold standard’ for collecting food intake data. However, given its time-consuming nature, it is labour-intensive, requires a high level of numeracy and literacy skills, and disrupts habitual day-to-day activities, which may ultimately impact on compliance.

Food diaries, also referred to as estimated food records, are similar to weighed food records except the quantification of foods and drinks are estimated as opposed to being weighed. Estimates are carried out using household measures, such as cups or spoons, food photographs or food models. The investigator

converts these estimations into weights that can be used to derive food and nutrient intake. In a study by Bingham et al. (1994) the accuracy of dietary assessment methods were investigated in order to identify the most appropriate tool for gathering nutrient intakes in large scale epidemiological studies. Weighed food intake (for 16 days) were compared to 24-hour recall (with and without portion size photographs), food frequency questionnaire, and 7-day food diary methods in 160 adult subjects over the course of one year. Findings highlighted that there were no significant differences in nutrient contents quantified by 7-day food diaries when compared to the weighed food method. The 7-day food diary and weighed intake had the strongest correlation coefficients when compared to the other dietary assessment methods. Bingham et al. (1994) concluded that the 7-day food diary was a practical and efficient tool for collating large scale dietary intakes, and has since been validated and implemented for use in the European Prospective Investigation of Cancer “EPIC” study (Day et al. 2001; McKeown et al. 2001).

As with the weighed food intake, food diaries are affected by error. The tendency of subjects to report food consumption close to those socially desirable is the main drawback of using food diaries, leading to underreporting (Cook et al. 2000). Some subjects can experience difficulties in writing down food and drink consumed or in describing portion sizes (Ortega et al. 2015). Processing information collected from food diaries is time consuming, however utilization of specific software programmes with comprehensive databases of foods and also common household measures of each food can ease the burden of dietary analysis considerably.

3.3 Hormonal Control – Biomarkers of Appetite

Several physiological changes, such as gut peptide concentrations, show direct relationships with appetite ratings or food intakes and can be used as biomarkers of appetite (Mattes et al. 2005).

Glucose has been at the centre of focus with regards to short term appetite regulation theories since the emergence of the glucostatic theory of eating more than 60 years ago (Mayer 1953). Spontaneous meal request is often preceded by a reduction in blood glucose utilisation, thus blood glucose has been proposed

as an appetite biomarker candidate. There is an abundance of evidence to support attenuation of post-prandial glucose and insulin following β -glucan consumption (EFSA 2011), yet more evidence is required with regards to the incretin response, in particular GLP-1. In the context of appetite and energy intake, to the researcher's knowledge post-prandial responses in GLP-1 have only been investigated in two studies (Juvonen et al. 2009; Hartvigsen et al. 2014).

3.3.1 GLP-1

GLP-1 has been shown to be a potent determinant of post-prandial insulin release that occurs following increases of blood glucose (Fehmann et al. 1995). As a result, GLP-1 is also essential for the regulation of post-prandial glycaemia. Consistent with GLP-1's incretin function, carbohydrates (CHO) are rapid and potent secretagogues which result in systemic increases of GLP-1 within 15 minutes of meal onset (Herrmann et al. 1995; Holst 2007;). When plasma glucose concentrations are within a normal fasted range, GLP-1 no longer stimulates insulin to cause hypoglycaemia, thus delays and protracts CHO absorption, increasing its satiating effects.

Controlled studies in humans that investigate the effect of β -glucan with regards to the incretin response are scarce, with a recent literature database search highlighting only two acute appetite studies quantifying post-prandial GLP-1 responses following β -glucan consumption (Juvonen et al. 2009; Hartvigsen et al. 2014).

Measurement of GLP-1 is complicated by low circulating concentrations in the blood. In peripheral venous blood from fasted healthy humans, plasma concentrations of total GLP-1 (intact peptide and its primary metabolite) are normally below 20 pmol/L, whereas the concentration of the intact/biologically active form of GLP-1 accounts for a mere fraction (usually 10–20%) of the total concentrations (Kuhre et al. 2015). Active GLP-1 (7-36 amide) is rapidly degraded by DPP-4 to GLP-1 (9-36 amide), which limits its biological half-life in the blood to 1–3 minutes (Steinert et al. 2016). This very short half-life complicates

investigations towards GLP-1's true satiating nature, thus total GLP-1 is a more achievable and sustainable marker.

3.4 Laboratory studies

Appetite studies are generally carried out in the laboratory setting in order to try to control for any environmental factors that may influence eating behaviour (Benelam 2009). Naturally, studies conducted in these artificial environments may lack external validity, yet given the nature of the setting, tightly controlled laboratory studies have high internal validity because they have the highest degree of sensitivity and control over both intervention and outcome measures (Blundell et al. 2010). For example, if VAS are administered by an investigator in the laboratory setting, he/she can ensure that the subjects complete the VAS at the appropriate times. This may not be guaranteed if the subject were to leave the experimental setting.

Development of concepts and how changes in foods can affect eating behaviour can be achieved in the laboratory setting. It is not until these have been established within a controlled environment that they can potentially be evaluated for applications outside the laboratory setting. Yet, there may be potential drawbacks to the external validity of free-living studies, such as errors in data collection methods. A particular example would be bias surrounding measurements of food intakes, where underreporting of energy intakes and mis-reporting of macronutrients is commonplace (Livingstone and Black 2003).

3.5 Test Meal Studies

Whilst Blundell and colleagues (2010) highlight the many considerations in interpreting data from test-meal studies, appropriate implementation of robust protocols will help maximise outcome measures from a repeated measures study design. Test meal studies are only considered useful when several outcome measures are investigated, particularly VAS and *ad libitum* energy intakes. Gregersen et al. (2008) highlight that it is not necessary for subjects to undergo prior diet standardisation before participating in acute satiety

studies when *ad libitum* energy intake is used as an outcome measure. They report that no effect of prior diet standardization was seen on the reproducibility of *ad libitum* energy intakes, however diet standardization did increase *ad libitum* energy intake significantly. Since prior diet standardization exerts a significant effect on *ad libitum* energy intake, such consideration should be acknowledged in relation to study design.

It is important to not lose sight on the fact that test meal studies will only define the acute effects of a particular food. Measuring energy intake after test meals is the most efficient way to identify satiating effects, along with changes in GI hormones which may or may not correlate with such feelings of satiation. Yet hormones will act as a marker of satiation only, since they do not always correlate with subjective feelings of appetite and energy intake (de Graaf et al. 2004).

3.6 Characterization of dietary fibre

Despite awareness of the diversity of dietary fibres and the extensive volume of work related to their effects on various physiological and metabolic outcomes, surprisingly little attention has been paid to consistently defining and reporting dietary fibre materials used in nutrition research. This is especially important for the translation of dietary fibre research into practice. For example, a wide range of proposed claims related to the health effects of dietary fibre have not been authorized by the European Union, largely due to inadequate characterization of the materials used in the supporting research (EFSA 2010). This reflects the recognition that evidence for claimed benefits of a given dietary fibre can only really be applied to that same dietary fibre or another with the same physiologically relevant properties.

The need to identify and replicate the exact materials used in nutrition studies is emphasized by the diversity of both dietary fibre properties and physiologic mechanisms of action, as seen in **Figure 8**. Adequate specification of dietary fibre at the levels shown in **Figure 8** will help ensure validity when comparing and combining studies in meta-analyses, and furthermore, to establish reliable, predictive structure-function relationships between specific fibres and fibre-containing foods with physiological effects.

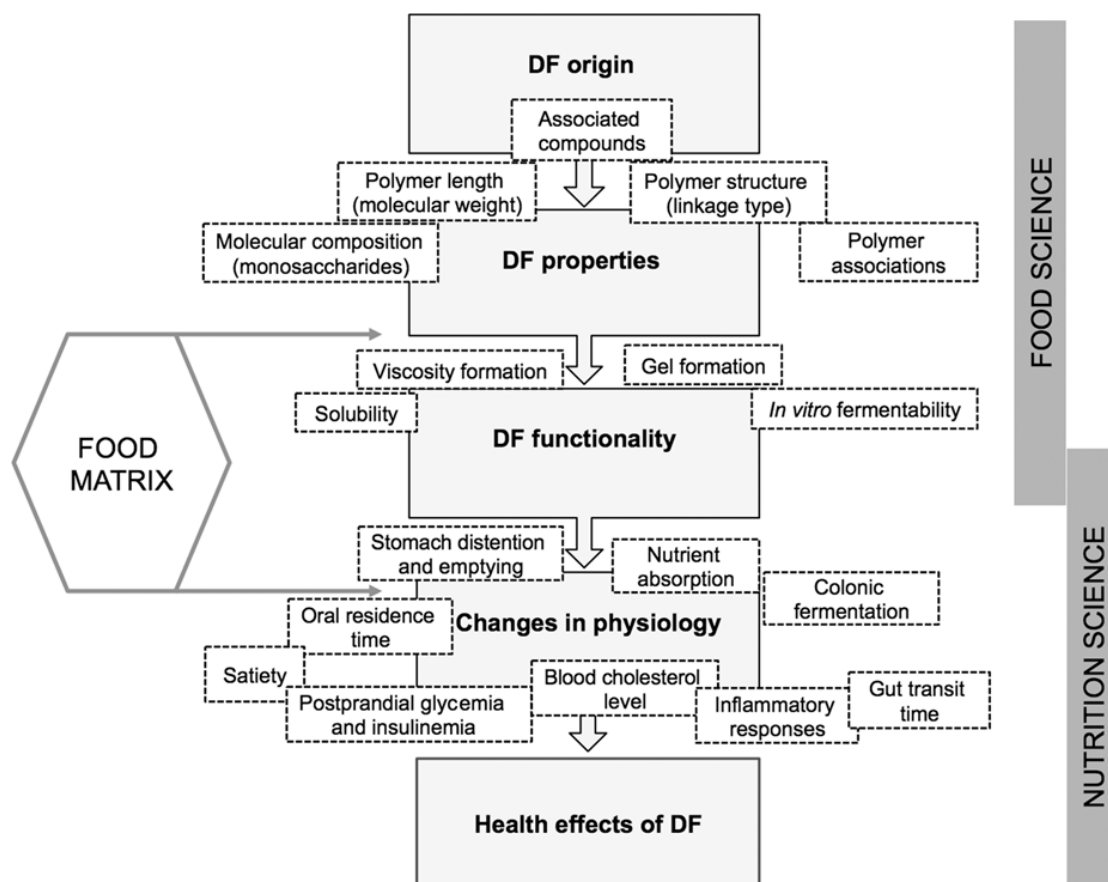


Figure 8. Properties, functionalities and physiological effects of dietary fibre (DF) relevant to health outcomes. Figure taken from Poutanen et al. (2018).

Simply detailing the name and origin of a given dietary fibre is not sufficient. Even if the assessment of nutritional physiological measurements are robust, omission of the physiochemical characteristics of the fibre under investigation will not explain what characteristic of the fibre is relevant, nor will predicting with confidence what other sources of dietary fibre may have the same effects (Poutanen et al. 2018). It is also important to recognise that because an increasing amount of dietary fibre comes from commercially manufactured dietary fibre containing ingredients and products, knowledge of structure-function relationships will help to develop foods with desired health benefits (Li and Komarek 2017).

Recently, Poutanen and colleagues (2017) highlighted the magnitude of the issues related to nutritional interventions with regards to poor reporting of fibre characterization; in their systematic review, which

focussed on dietary fibre properties in relation to energy intakes and appetite outcomes, 75% of otherwise eligible articles were excluded due to lack of information regarding fibre details (other than fibre source and name). However, in the few studies that did include adequate dietary fibre characterization, the approach and methods used for characterizing fibres, and standards of reporting such features, varied widely (Poutanen et al. 2017). Thus, improved dietary fibre characterization and reporting standards are required to, i) maximize the value of published research, and ii) develop better mechanistic knowledge and reliable prediction of the effects of specific dietary fibres in nutrition.

3.7 Confounders in acute appetite studies

Confounding is often referred to as the mixing of effects wherein the effects of an intervention on a given outcome are mixed in with the effects of an additional factor (or more) resulting in a distortion of the true relationship (Weiss 2006). Confounding factors may mask an association, or more commonly, falsely demonstrate an apparent association between treatment and outcome when no real association between them exists (Skelly et al. 2012).

Table 4 outlines key experimental evidence which surrounds general confounders which may be encountered in appetite studies, and how they may be controlled for when designing appetite and eating behaviour-based research studies. These potential confounders were addressed with respect to the implications for the current study's design and protocols adopted.

Table 4. Evidence of physical, behavioural and properties of foods which may confound acute appetite research and recommendations for study design

Confounder	Evidence	Recommendation for study design
PHYSIOLOGICAL		
Bodyweight / body composition	<p><i>Differences between lean and obese individuals:</i> Obese subjects have generally higher energy requirements than lean subjects (Benelam 2009).</p> <p>Obese subjects have higher circulating ghrelin (Buss et al. 2014) and reduced GLP-1 (Madsbad 2014) and PYY (Karra et al. 2009). Mutations in leptin gene identified in obese subjects (Clement et al. 1998). No acute appetite studies have been identified that include subjects who are healthy underweight.</p>	<p>Measurement of an appetite response may vary according to the body composition or overall BMI of an individual. It cannot be assumed that an outcome observed in a group of lean people will be replicated in a group of obese.</p> <p>Studies should choose to include lean, overweight or obese subjects only. In some cases, differences between these populations may be of interest, thus satiety and energy intake maybe be compared in lean, obese or overweight subjects.</p>
Gender	<p><i>Males:</i> Generally have larger lean mass and weigh more (higher BMR) and tend to consume more in the <i>ad libitum</i> meal setting.</p> <p><i>Females:</i> Have lower energy requirements and are likely to eat less (Benelam 2009). Hormonal fluctuations in females of childbearing age – females report increase in food cravings prior to menses and during menses (Rozin et al. 1991), yet McVey et al. (2012) showed that cravings between menstrual cycle phases did not differ in non-disordered eating females.</p>	<p>Aim to include an equal number of males and females in study cohort.</p> <p>The phase of the menstrual cycle may not impact on eating behaviour and food choices as previously thought. Therefore, as concise evidence surrounding the influence of the menstrual cycle on human energy balance is still largely uncertain, menstrual cycle influences on food intakes should be acknowledged. There are considerable methodological problems in investigating PMS (Dye and Blundell 1997).</p>

Age	<p><i>5th and 6th decade of life:</i> Post-menopausal females have higher calorie intakes compared to women in menopausal transition. Hunger is reported to increase during menopausal transition and remain higher in postmenopausal years (Duval et al. 2014).</p> <p><i>With advancing age:</i> “anorexia of ageing” GE slows (Nieuwenhuizen et al. 2010). Fasting levels of ghrelin are lower (Di et al. 2008) and CCK sensitivity increased (Parker and Chapman 2008; de Boer et al. 2012).</p> <p><i>Increased prevalence of acute disease:</i> Anorexic effect of pro-inflammatory cytokines. Chronic pain and medications reduce appetite (Langhans 2007). Reduced senses – taste, smell and vision loss (Pilgrim et al. 2015). Lower energy requirements (Goodpaster et al. 2006). SSS declines with age (Benelam 2009).</p>	<p>Appetite studies should recruit individuals of a particular age group, avoiding individuals who are ≥ 50 years.</p>
Disease status	<p><i>Disease and medications may:</i></p> <p>Activate the inflammatory response, thus altering metabolic requirements (Wang and Ye 2015). Impair absorption of nutrients (Brownie 2006). Reduce motivation to eat (Pilgrim et al. 2015).</p>	<p>Unless a medication or disease state is of particular interest, individuals who are taking medications are normally excluded from participating in appetite studies.</p>

BEHAVIOURAL

Dietary restraint	<p><i>Restrained eaters:</i> Restrict food intake in order to maintain or lose bodyweight Control of eating is lost ('disinhibition') and possible binge eating episodes</p>	<p>The Dutch Eating Behaviour Questionnaire (van Strien et al. 1986) or the Three Factor Eating Questionnaire (Stunkard and Messick 1985) should be completed by all subjects in order to determine levels of dietary restraint. Restrained eaters should be excluded from participation, unless restrained eating is of particular focus to the study.</p>
Habitual diet	<p>Control of diet before a study may be important for those who are not in energy balance, ie., obese subjects (Livingstone et al. 2000) However, standardizing the diet of subjects prior to a study has been reported to not improve the reproducibility of <i>ad libitum</i> energy intakes (Gregersen et al. 2008).</p>	<p>Instruct subjects to avoid consuming extremely small or large evening meals the night prior to study sessions. Ask subjects to consume the same evening meal prior to attending various study sessions.</p>
Smoking	<p><i>Nicotine is an appetite suppressant:</i> Increased levels of acetylated ghrelin found in individuals who stopped smoking (Koopmann et al. 2015). Smokers have lower BMI than non-smokers (Pistelli et al. 2009). 80% of smokers who quit gain weight (Koopmann et al. 2015).</p>	<p>Individuals who smoke should be excluded from appetite studies, unless smoking status is of interest.</p>
Alcohol consumption	<p><i>Alcohol may stimulate appetite:</i> Alcohol preload studies show alcohol can increase high calorie food consumption in <i>ad libitum</i> food settings (Yeomans 2010; Christensen et al. 2015).</p>	<p>Subjects are asked to abstain from alcohol consumption the day prior to and on the day of the study.</p>
Physical activity	<p><i>Habitual physical activity may improve appetite control:</i></p>	<p>Subjects should be asked to refrain from strenuous exercise on the day prior to the test day in order to</p>

	<p>PYY and GLP-1 respond differently to exercise in males and females following various exercise intensities (Hazell et al. 2017).</p> <p>Systematic review by Schubert et al. (2013) reports energy intake after acute exercise was greater in active individuals than less active individuals.</p> <p>In contrast, systematic review findings from Donnelly et al. (2014) suggest that regardless of the level of physical activity, there was no consistent effect on acute energy intake</p>	<p>standardise test conditions. Subjects should be instructed to avoid exercising the morning of the study and requested to travel to the research centre by the least strenuous means (car, bus, train, light walking).</p>
Caffeine	<p>Increasing caffeine consumption is associated with lower mean weight gain and energy intake (Lopez-Garcia et al. 2006).</p> <p>Caffeine may have an acute, mild appetite suppressant effect, however there is conflicting evidence.</p> <p>Schubert et al. (2013) reported no effect of caffeine on GE or acute energy intakes.</p>	<p>Subjects are asked to abstain from caffeine containing foods and drinks (particularly coffee) the day before and morning of the study.</p>
Prior knowledge regarding test meals	<p>Expected satiety has been shown to affect appetite responses (Brunstrom et al. 2011).</p>	<p>Blinding subjects to the study is recommended. Subjects should not be aware of the alterations made to the meals prior to the test meal to avoid altering their behaviour</p>
PROPERTIES OF FOODS		
Macronutrient composition	<p><i>Hierarchy of satiety effect:</i> Protein > carbohydrate > fat</p> <p><i>Protein:</i></p>	<p>Macronutrient content should be matched in test foods, unless they are the variable of interest.</p>

	<p>Out of 14 randomised trials comparing high protein preloads to at least one other macronutrient, 11 found protein significantly increased subjective ratings of satiety (Halton and Hu 2004).</p> <p><i>CHO:</i> Difficult to distinguish the physiological effects of refined CHO on satiety and psychological effects of sweetness</p> <p><i>Fat:</i> Slows GE and stimulates the release of ghrelin (Gentilcore et al. 2006). Increases palatability of foods. Satiating effects weaker than either protein or CHO.</p>	<p><i>Ad libitum</i> foods should not contain a high fat content – more food may be consumed than normal as a result.</p>
Palatability	<p><i>Appearance and palatability of food:</i></p> <p>Determine the magnitude of voluntary nutritional intake (Blundell and Stubbs 1999). Palatability of a food has a positive effect on the amount eaten (de Graaf et al. 1999). Potentially can override other mechanisms of food intake regulation.</p>	<p>Since ‘liking’ and ‘disliking’ foods can heavily influence food intake, all test foods/meals should be piloted before commencing appetite studies to ensure that foods are palatable.</p>
Portion size	<p><i>Cognitive and sensory influences:</i></p> <p>Young and middle-aged adults consume more energy when provided with a larger portion meal (Flood et al. 2006; Rolls et al. 2006) Using larger bowls and spoons increased calorie consumption of energy dense snacks (Marchiori et al. 2012)</p>	<p>All meals / test foods should be piloted before study commences. Volumes and portion sizes in appetite studies should be matched where possible (unless variable of interest). Effort should be made to use the same eating utensils throughout duration of the study.</p>

3.8 Development of test breakfast

3.8.1 OatWell™ oat bran powder

Oats are traditionally consumed as flakes and porridge, but since the discovery of the health benefits of β -glucan, the use of oats has expanded to many food categories, such as bakery products, healthy snacks and drinkable products (Rasane et al. 2015). From a technological perspective, the degradation of β -glucan is often favoured by processing to enhance product texture and sensory (organoleptic) properties. However, uncontrolled degradation may spoil the product structure and adversely affect the health benefits (Ahmad and Kaleem 2018).

From a product perspective, it is important to determine a minimum dose of a high cost ingredient which will still deliver physiological benefits. From a research perspective, products are initially tested at high doses to identify any possible beneficial effects. β -glucan has been investigated frequently at doses of 5 g or more (Beck et al. 2009a; Juvonen et al. 2009; Lyly et al. 2010; Juvonen et al. 2011; Pentikäinen et al. 2014). Beneficial effects aside, consumption of foods (oats and barley) to consume these amounts of β -glucan is not acceptable as a regular eating pattern, primarily due to the volume required. For example, there are 3.6 g β -glucan per 100 g of Quaker traditional oats. Keeping in mind that a standard portion of porridge is around 40 g, an individual would need to consume around 140 g of oats to ingest 5 g of β -glucan, which is approximately 3 ½ - 4 bowls of porridge. Not only is this an unpalatable volume, it would also be calorific (around 520 kcal from the oats alone, without milk or toppings). Therefore, focus has turned to using concentrated oat bran products to deliver β -glucan to the diet in adequate doses, without the addition of excess calories to the diet.

OatWell™ oat bran powder is an example of a versatile functional fibre ingredient produced by DSM Nutritional Products Ltd. (Kaiseraugst, Switzerland), currently used in foods and beverages worldwide as a potential solution to increase fibre intake. OatWell™ oat bran powder is concentrated β -glucan derived from non-GM Swedish oats, produced by a chemical-free, aqueous, enzymatic process. The final product is a fine, beige coloured powder with a caloric value of 2.74 kcal per gram of ingredient. It is a source of

28% oat soluble β -glucan fibre by weight and contains protein, fat and low carbohydrate (**Table 5**). Oat β -glucans from different sources have a wide range of MWs, which are heavily influenced by processing conditions. Tosh et al. (2010) previously determined the MW of OatWell™ oat β -glucan to be 2.213×10^6 g mol⁻¹. The oat bran powder thickens and stabilises emulsions, which results in a smooth texture, possessing strong water-binding properties.

OatWell™ was the test substance used in several of the 28 randomised controlled trials that featured in the most recent meta-analysis which focussed on β -glucan's cholesterol lowering effects (Whitehead et al. 2014). Wang and Ellis (2014) reviewed a number of studies using OatWell™ that demonstrate that high viscosity β -glucan reduced not only cholesterol but post-prandial glucose levels. Given the present evidence of OatWell™ in studies investigating cholesterol and blood glucose, it is important to identify its role in satiety and satiation studies.

Table 5. Typical nutritional composition per 100g of OatWell™ derived from natural levels in oats.

Nutritional product information provided by OatWell™ (OatWell™ 2014).

	OatWell™ oat bran powder
Energy	274 kcal
Total dietary fibre	52 g
<i>β-glucan soluble fibre</i>	28 g
Carbohydrate	9 g
Protein	23 g
Total lipids:	5 g
<i>Includes saturated FA</i>	1 g
<i>Polyunsaturated FA</i>	2 g
<i>Monounsaturated FA</i>	2 g

3.8.2 Test breakfast

The aim of designing the test breakfast was to provide a breakfast meal to all study participants that would contain 4 g oat β -glucan. The practicality of the breakfast and its organoleptic acceptance were also taken into consideration. With respect to meal design, the development of the test breakfast was produced using familiar and accessible ingredients that would firstly, not be costly, and secondly, would be commonly consumed by the Scottish population. After reviewing existing literature of comparable cross-over trials, caloric content of test breakfasts ranged from 218-363 kcal (Vitaglione et al. 2009; Rebello et al. 2013; Rebello et al. 2014), which varied depending on subjects recruited to each study. Providing a breakfast ~218 kcals would be only 10 % of daily caloric intake for female adults (2000 kcal per day), therefore, the researcher aimed to design test breakfasts between 300-400 kcals to align with current recommendations (NHS 2017). The researcher considered several options when designing the test breakfast. **Table 6** highlights the ingredients considered, along with the advantages and drawbacks of each. The breakfast chosen for use in this research was Rice Krispies cereal (Kellogg Company, Manchester, UK), served with semi-skimmed milk (1.8 % fat) and Greek-style yoghurt (Tesco Groceries, Edinburgh, UK). These ingredients received the most positive feedback in terms of taste and appearance when piloted in a small number of subjects (n=5). During the recipe design phase, the viscous nature of the OatWell™ became very apparent. The consistency of the yoghurt after being mixed with the oat bran powder visibly thickened over time, and when mixed with a spoon was elastic-like in appearance. To achieve 4 g of β -glucan, 14.6 g of OatWell™ oat bran powder was required. The researcher experimented with manipulating volumes of oat bran powder into both the Greek-style yoghurt and Rice Krispies cereal. The most palatable method was to split the oat bran powder equally between both ingredients, thus 7.3 g of oat bran powder was added to the Greek-style yoghurt and 7.3 g added to the Rice Krispies cereal. In order to increase the palatability of the cereal, and to reduce the unpleasantness of ‘thick’ milk, milk was kept separate from the Rice Krispies cereal until it was time to commence consumption of the study breakfasts. This also prevented the Rice Krispies from becoming ‘soggy’ and unappealing.

Table 6. Breakfast design considerations

Breakfast Meal (ingredients)	Advantages	Disadvantages
Pancakes – <i>flour, water, eggs, OatWell™</i>	Oat bran powder was concealed inside the product well.	Heat from frying pan may reduce functional properties of β -glucan. Time consuming to prepare.
Fruit smoothie – <i>orange juice, bananas, apple, OatWell™</i>	Quick and easy to prepare. Palatable. Oat bran powder was relatively undetectable.	GE of liquids is faster than it is for solids. Fructose absorbed quicker, potential glucose spike.
Scrambled eggs – <i>eggs, milk, butter, OatWell™</i>	Oat bran powder was concealed inside the product well.	Time consuming. Recipe contained too much protein (most satiating macronutrient). Heat may reduce functional properties of β -glucan.
Rice Krispies cereal – <i>Rice Krispies, semi-skimmed milk, OatWell™</i>	Oat bran powder could be concealed in cereal easily.	Cereal becomes ‘soggy’ after a few minutes.
Natural yoghurt, OatWell™	Quick and easy to prepare.	After mixing, yoghurt appeared beige in colour.
Greek-style yoghurt, OatWell™	Quick and easy to prepare. Thicker consistency masked oat bran powder better than natural yoghurt.	Following mixing, viscosity visually increases ‘bitter’ taste.

Chapter 4

4.0 Effects of oat β -glucan consumption at breakfast on ad libitum eating, appetite, glycaemia, insulinaemia and GLP-1 concentrations in healthy subjects (Study A): Research Methodology

4.0.1 Study Pre-requisites

Before commencing the study, the researcher obtained ethical approval, adult venesection (NHS Lothian) and first aid certification. Since there were no existing protocols at Queen Margaret University for blood sampling from an IV cannula (with saline flush), this added a significant delay to gaining ethical approval. Along with guidance and assistance from Caroline Gibson (Queen Margaret University, Registered Nurse) and Simon Holmes (Queen Margaret University, Registered Radiographer) it was imperative for the researcher to develop a protocol to carry out this method for serial blood sampling before ethical clearance was permitted. Ethical approval for this research study was granted by Queen Margaret University Research Ethics Panel on 8th December 2015.

4.1 Study design

This experimental study was a double-blind, randomized, placebo-controlled cross-over study. Each subject acted as their own control, therefore minimising the impact of confounding factors (Lovegrove et al. 2015). All subjects visited QMU to be screened and their eligibility checked. Eligible subjects were required to attend two study mornings (approximately three and a half hours in duration each) with at least seven days (but no more than 30 days) apart to allow for adequate washout between the two different treatments, in accordance to Blundell et al. (2010). Subjects were required to attend sessions on the same day of the week as to reduce the influence of lifestyle variances on outcome measures.

The current study comprised of two one-day testing periods, which followed a typical acute appetite study design (Blundell et al. 2010). The study aimed to assess the impact of β -glucan consumption on markers of appetite, satiation and satiety in healthy normal-overweight adults.

4.1.1 Eligibility of subjects

This study was open to healthy men and woman aged 18-50 years old, with a BMI between 20 - 29.9 kg/m², who normally consumed breakfast. It was essential that subjects were able to provide informed consent to participating in the study and agreed to follow all pre-study requisites, such as abstaining from strenuous exercise, consuming alcohol and caffeine drinks 24 hours prior to study mornings, in addition to undergoing 10 hours of fasting. Given the nature of the study, it was also important that subjects did not show signs of dietary restraint, impaired handling of glucose or had reduced haemoglobin, all of which were measured during screening sessions. Subjects were not permitted to take part in the study if they had CVD or GI disease, or were taking medications which may alter appetite. Individuals who were dieting, had food allergies to any of the test meal ingredients (wheat, dairy), vegetarians or vegans, individuals with needle phobia or had recently donated more than 300 mL of blood three months prior to the screening visit were excluded. Postmenopausal, pregnant or breastfeeding females were not eligible.

4.1.2 Statistical power

A comprehensive literature search of comparable cross-over trials showed a decrease in energy intake at *ad libitum* lunches of between 80 to 170 kcal, which varied depending on a number of factors, such as dose of ingested β -glucan, inter-meal intervals, subject characteristics, and test-meal compositions. For example, in a study by Vitaglione et al. (2009) a 3 g β -glucan intervention at breakfast reduced *ad libitum* lunch energy intake after 3 hours by 170 kcal, whereas Rebello et al. (2016a) report an 85 kcal deficit at an *ab libitum* lunch following 2.68 g of β -glucan consumption at breakfast followed by a 3 hour inter-meal interval. Standard deviations were not reported in these studies, but could be derived from reported p-values and number of subjects with consideration of the statistical model applied. In addition, previous research by Wanders et al. (2011) indicated that the standard deviation of decreases in *ad libitum* energy intake after

nutritional interventions ranged from 130 to 220 kcal, which was in agreement with the standard deviations above.

Using an average standard deviation of 200 kcal and assuming a conservative decrease in energy intake of 100 kcal, the resulting expected effect size was 0.5. Therefore, the resulting necessary sample size was $n=32-34$ (one sample t-test, $\alpha=5\%$, power of 80%). The sample size software used was nQuery Advisor® (version 7.0, Statistical Solutions Ltd., Cork, Ireland). A minimum of 36 subjects were required to allow for additional variability and potential missing data.

4.1.3 Recruitment

Subjects were recruited to take part in this research study between February 2016 and March 2017 via convenience sampling methods.

The purpose of recruitment was to, i) screen and check the eligibility of subjects, and ii) test an adequate number of eligible subjects to fulfil the sample size estimated to meet the outcomes of this study. There were no monetary incentives offered to subjects. The researcher did however offer nutritional feedback from food diaries and provided subjects with information gathered at the screening session. Food used for the test breakfasts and *ad libitum* lunches were purchased by the researcher. DSM Nutritional Products Ltd. (Kaiseraugst, Switzerland) provided the study with OatWell™ oat bran powder and £2800 towards analytical costs.

The researcher initially made informal enquiries by telephone and email to local community centres and groups in and around the Musselburgh area. When positive feedback was received from these locations, posters and leaflets were distributed. All recruitment material were approved by Queen Margaret University Research Ethics Panel. Material included information regarding the study that was suitable for the lay public, and contained the researcher's contact details (address, email and office telephone number). In addition to posters and flyers, an advertisement was also circulated throughout Queen Margaret

University's email system to all staff and students periodically for approximately 18 months. During the summer months, responses from students and local areas diminished. In order to increase interest and find more subjects, a press release was posted on the Queen Margaret University website and social media pages. After the researcher received notice of interest from potential subjects, subject contact details were recorded in a password protected Microsoft Excel file. This ensured all subjects were contacted and followed up in a timely manner, which was at times challenging when high numbers of responses were received, i.e., following the press release.

In order to successfully complete the study, eligible subjects were required to attend the screening session (up to 60 minutes in duration) and two study mornings (around three and a half hours each morning). Assessing changes in hormones required serial blood sampling, thus individuals who had needle phobia did not participate in the study.

4.2 Screening session

Interested individuals attended a consultation session with the researcher to discuss eligibility for participation, followed by specific screening tests which determined their suitability. If the subject was found to meet the inclusion criteria, they were given the participant information sheet (**Appendix 2**) to read over for a second time and given time to ask relevant questions. Before any screening tests were performed, the subject gave signed consent.

To determine if potential subjects met the full study criteria, an informal screening interview was performed. A series of questions were asked with regards to:

- Date of birth
- Smoking status
- History of metabolic/ CVD or GI disease

- Allergies or food intolerances
- Medications or supplements
- Breakfast habits (do you skip breakfast?)
- Needle phobia
- When did you last donate blood?
- Weight stable in the past 3 months?

Anthropometry was then measured in order to determine BMI and WC (described in full, **section 4.5**). A single-use lancet was used to take a finger-prick sample of blood to check fasting blood glucose and haemoglobin. Subjects with a fasting blood glucose level of > 5.6 mmol/L (ADA 2016) or males and females with haemoglobin of < 130 or < 120 g/L (WHO 2011), respectively, were not eligible to take part. Dietary restraint refers to the tendency to restrict food intake in order to control bodyweight. It was not realistic to include subjects in this study who demonstrated this behaviour, as research outcomes could be bias due to a conscious decision to limit energy intake, which may also be followed by a period of binge eating. These behaviours would not reflect true feelings of appetite. Subjects were provided with a Dutch Eating Behaviour Questionnaire (DEBQ, **Appendix 3**) in order to measure their dietary restraint. The DEBQ was developed by van Strien et al. (1986) and is used extensively in appetite research due to its internal and external validity. The questionnaire consists of 33 questions; 10 questions to determine dietary restraint, 13 questions to determine emotional eating and 10 questions to determine externally induced eating. Subjects were required to respond to each question with either ‘never’, ‘seldom’, ‘sometimes’, ‘often’ or ‘very often’. With regards to dietary restraint, a threshold score of ≤ 2.97 was the cut-off score for subjects with a BMI ≥ 26 and ≤ 2.43 for those with a BMI < 26 (van Strien et al. 1986) which is indicative of an acceptable level of dietary restraint.

Finally, a physical activity questionnaire was completed by subjects. The Scottish Physical Activity Questionnaire (SPAQ; Lowther et al. 1999) was used to aid 7-day total physical activity recall, including

physical activity at work, travelling to work and leisure time. The SPAQ has been shown to hold good test-retest reliability, concurrent validity, and limited criterion validity (Lowther et al. 1999). The SPAQ was used to determine physical activity levels of all subjects (**Appendix 4**). Subjects were asked to complete the SPAQ on two more occasions (during their study morning sessions) to check compliance to the study; subjects were asked to avoid strenuous exercise 24 hours prior to study morning testing.

4.3 Study morning protocol

On each individual test day subjects were required to arrive at QMU following an overnight fast of 10 hours. This was to ensure subjects attended the study morning in the similar state of hunger on each occasion. As highlighted in **Table 4 (section 3.7)**, diet and exercise habits may be potential confounders, therefore subjects were instructed to abstain from strenuous exercise, alcohol and all caffeine-containing beverages and foods 24 hours prior to and during the study day. Subjects were also asked to consume the same (or similar) evening meal prior to study sessions. In addition, subjects were asked to wear light clothing and if possible wear a short-sleeved top to gain easy access to blood sampling sites. Weight, WC and BP were measured on the subject's arrival and the first VAS was administered. Subjects were then asked to relax and lie down on a clinical plinth before an IV cannula was inserted into a vein in the antecubital fossa area. After patency of the IV was checked, fasted blood samples were withdrawn. Subjects were then escorted to the meal test room which was an open plan kitchen space. The researcher instructed that the subject should remove the covers from their study breakfast and begin eating only after she left the room. The subject completed a palatability questionnaire whilst consuming their breakfast, which was then turned face down to prevent the researcher from reading. The researcher remained outside the room until the subject finished eating and recovered their bowls. Bowls were recovered because it would be easy to detect if OatWell™ oat bran was present; OatWell™ oat bran powder had a yellow-beige colour when added to the test breakfast ingredients, thus would un-blind the researcher to the study. Time was noted immediately after the subject finished eating, which was used to calculate the timings of subsequent VAS

and blood samples taken throughout the morning. After a period of 150 minutes following breakfast, subjects returned to the eating area and were served and *ad libitum* lunch. During the inter-meal period VAS were completed every 15 minutes and blood samples were withdrawn at 30, 60 and 90 minutes following breakfast consumption. **Figure 9** summarises measurement time points on test meal days.

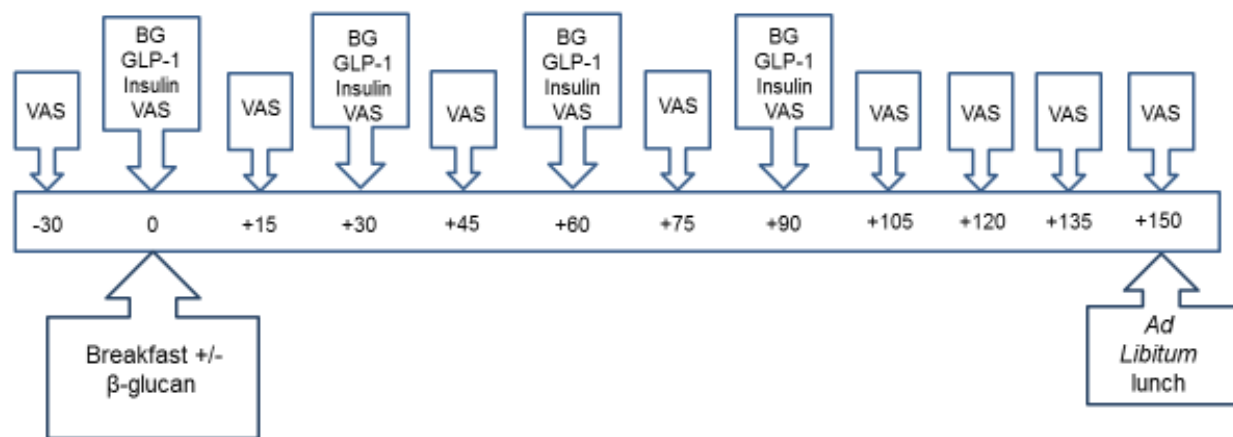


Figure 9. Measurements taken during the course of each study morning.

BG, blood glucose; GLP-1, glucagon-like peptide-1; VAS, visual analogue scale.

4.3.1 Test breakfast

The breakfast consisted of Kellogg's Rice Krispies cereal (Kellogg Company, Manchester, UK), with semi skimmed milk (1.8 % fat) and Greek-style yoghurt (Tesco Groceries, Edinburgh, UK). Four grams of high MW oat β -glucan (from 14.6 g of OatWell™ Original Powder, DSM Nutritional Products Ltd., Kaiseraugst, Switzerland) was split between the cereal and Greek-style yoghurt to improve palatability of the breakfast. For this, 7.3 g OatWell™ powder was mixed with Greek-style yoghurt and 7.3 g OatWell™ powder was

mixed with dry Rice Krispies in a bowl before semi-skimmed milk (150 mL) was poured over the Rice Krispies by the subject immediately before commencing the meal.

The breakfasts were matched for their protein, fat and carbohydrate contents. In order to accommodate for the energy content of the OatWell™ oat bran powder, the Greek-style yoghurt was reduced by 10 g in the intervention breakfast. In order to adequately match protein and carbohydrate contents of both breakfasts, 28 mL of PROmilk50 (ready-to-drink vanilla protein milk, MyProtein, Cheshire, UK) was added to the control breakfast. The ingredients and macronutrient content of each breakfast is shown in **Table 7**.

Subjects received their test breakfast in a random order to avoid an ‘order effect’ and thus potential bias of results. A researcher who was not involved in the study was responsible for assigning the order of the two breakfasts (with and without oat β -glucan) using a random number generator (Microsoft Excel). Subjects were required to finish the breakfast within 10 minutes and to rate the palatability of both breakfasts using a VAS.

In order for the study to be double-blinded, the researcher was assisted by undergraduate students. Students followed a strict set of instructions for how to correctly prepare the test breakfasts, particularly with regards to how to incorporate the OatWell™ oat bran into the intervention breakfast. The same bowls, cutlery and glasses were used throughout the study duration and subjects consumed breakfast in the same location. If there were more than one subject attending the same study morning, they would be positioned at opposite sides of the room so that they were unable to see the other subject’s breakfast. Subjects were not permitted to talk whilst they ate breakfast and instructed to consume breakfast within 10 minutes.

Table 7. Ingredients, energy and macronutrient composition of the breakfasts

	Control	β -glucan
Ingredients		
Kellogg's Rice Krispies (g)	30	30
Semi-skimmed milk (1.8% fat) (mL)	150	150
Greek-style yoghurt (g)	90	80
Protein milk (mL)	28	-
OatWell™ oat bran (g)	-	14.6
Nutrient content		
Total energy (kcal)	319.8	329.1
Fat g (% of total energy)	11.6 (33)	11.3 (31)
Carbohydrate g (% of total energy)	39.7 (50)	39.0 (47)
Protein g (% of total energy)	13.8 (17)	13.9 (17)
Fibre (g)	0.3	7.9
β -glucan	0	4
Weight (g)	298.0	274.6

Nutritional information was taken from nutrient declarations present on product food labels.

4.3.2 Sensory attributes of test breakfasts

Visual appeal, smell, taste, aftertaste and palatability were measured by using a VAS questionnaire (**Appendix 5**) administered to subjects before they began to consume breakfast. The words 'bad' and 'good' were anchored at opposite ends of a 100 mm line. Subjects were required to mark on the line where they

rated their breakfast for each of the five sensory characteristics. Subjects were encouraged to complete the questionnaire as honest as possible and ensured that their feedback would not influence the study.

4.3.3 Eating variables

At the start of each study morning, the researcher carefully explained the instructions for completing the VAS ratings. Subjects were administered VAS 30 minutes before breakfast, immediately before breakfast and then every fifteen minutes during the inter-meal period. The current study used five questions; How hungry do you feel at this moment? How full do you feel at this moment? How strong is your desire to eat at this moment? How satiated are you at this moment? How much do you think you could (or would want to) eat right now?

Subjects recorded their feelings on paper questionnaires which were administered by the researcher (**Appendix 5**). After each individual VAS was completed, the questionnaire was removed from the subject to prevent them from referring to their previous ratings. To ensure valid and reliable results, subjects were urged to concentrate on each individual question, and were allocated enough time to spend on their response. Subjects were not permitted to discuss their VAS ratings with other participants. After completion of the study, the researcher measured all VAS with a standard ruler in order to quantify ratings into millimetres.

4.3.4 Energy intakes

Ad libitum energy intake (calories) of the meal consumed during the study morning was the primary outcome measure of this study. The presence of a variety of food cues has been shown to delay satiation and stimulate interest in different foods, ultimately increasing food intake (Hetherington et al. 2006). As mentioned previously, it has been reported that individuals will consume more kcals *ad libitum* when offered a wide range of foods, therefore a single-item lunch was used in this study.

The lunch given consisted of a ham sandwich, made from white medium sliced bread (approximately 40 g per slice, Hovis medium soft white, High Wycombe, UK), butter (10 g per slice, Countrylife, Surrey, UK)

and sliced cooked ham (approximately 45 g per sandwich, Tesco Groceries, Edinburgh, UK). The single-item food was a typical item consumed by subjects for lunch. Nutritional composition of the *ad libitum* meal (per 100 g) is shown in **Table 8**.

The sandwiches were cut into four iso-caloric pieces and served in excess to the subject after being weighed. Sandwiches were served in excess to reduce the subject's awareness to the amount of food eaten. Water was offered to the participant to drink. Subjects were instructed to eat and drink until they felt 'comfortably full'. Subjects were not aware that *ad libitum* energy intake was an outcome measure of the study. Knowing this may have influenced the subject's eating behaviour. As with the study breakfast, a palatability questionnaire was completed by the subject to identify sensory perceptions of the meal. It was important to receive feedback since palatability and 'liking' of foods heavily influences meal termination (Blundell et al. 2010). Once the subject had left the eating area, the researcher recorded the amount eaten and drank by weighing leftover food in order to determine the capacity of food consumed and time taken for each subject to complete their meal. Weighing scales were used to determine weights to two decimal places and a stopwatch was used to record eating time. Weight of uneaten food was subtracted from the weight of food given before caloric intakes were calculated for each subject at each visit.

Table 8. Macronutrient composition of *ad libitum* lunch, per 100g

<i>Ad Libitum</i> sandwiches (per 100g)	
Total energy (kcal)	242.7
Fat (% of total energy)	11.7 (43)
Carbohydrate (% of total energy)	23.9 (39)
Protein (% of total energy)	10.3 (17)
Fibre (g)	1.3
Salt (g)	1.2

Daily energy intake was a secondary outcome measure. Subjects were asked to complete a food diary the day prior to the study, the afternoon and evening of the study session (post study intake) and day after the study. Before starting the study, subjects were given guidance from the researcher on how to record their food intakes using the food diary. Along with detailed instructions inside the food diary, the researcher also verbally explained how the subject should describe food portion sizes in order to increase the accuracy of food quantities recorded. Food diaries were either emailed or posted back to the researcher, or if possible handed back to the researcher in person during the study sessions. Energy intakes were calculated using Nutritics® nutritional dietary analysis software (version 4.0, Nutritics Ltd., Dublin, Ireland). Energy consumed during the study (test breakfast and *ad libitum* energy intake) was added to post-study energy intakes to attain daily caloric intake.

4.4 Blood sampling

The timepoints for blood sampling were heavily influenced by ethical constraints. Queen Margaret University ethics committee allowed the researcher to withdraw a maximum of four blood samples per subject per session. With this in mind it was important that the researcher considered incretin hormone post-prandial peaks in order to sample around appropriate time points. Both GLP-1 and glucose peak around 30 minutes post-prandially (Lim and Brubaker 2006; Korakianiti et al. 2014) and insulin at 60 minutes (Robbins et al. 1987), therefore blood was withdrawn in a fasted state (0 minutes), and again 30, 60 and 90 minutes following breakfast consumption.

A large vein in the antecubital fossa was the preferred insertion site for the IV cannula. The site was swabbed with sterile water before insertion and a 3M™ Tegaderm™ (Berkshire, UK) transparent film was placed around the site to hold the cannula in place. The cannula (Venflon™ 23 gauge, Plymouth, UK) remained patent throughout the duration of serial blood sampling. Simon Holmes, a qualified radiographer certified in IV cannulation inserted the IV cannula for all subjects.

All blood samples were drawn using standard aseptic technique at 0 (fasted), 30, 60 and 90 minutes post-breakfast (10 mL x 4) by the researcher who was qualified in adult venesection. As per protocol, there was a maximum of two cannulation attempts made at gaining access to the subject's veins. If patency of the IV cannula was not achieved, the subject was asked to reschedule the session. If it was unlikely that the IV cannula could be inserted (inaccessible veins), the subject continued with the study despite no blood withdrawal. Quite often female subjects had small veins which meant the IV cannula would not remain patent. The IV cannula was carefully removed by the researcher following the final blood sample and a sterile cotton pad was pressed against the puncture site to stem the blood flow.

Glucose was measured via finger-stick blood sampling using a handheld Accu-Chek® Aviva monitor (Roche Diagnostics, UK). An alcotip pre-injection swab (Universal™ 70% isopropyl alcohol, Oxford, UK) was used to clean the puncture site. Using a single-use lancet (Accu-Chek Safe T Pro Plus, Roche Diagnostics, UK), blood was obtained from the side of the subject's finger. The side of the finger was a less painful site to sample from. The researcher avoided the thumb and index finger for sampling sites as these are the most frequently used digits and have a more sensitive nerve supply (WHO 2010). The researcher also avoided applying any unnecessary pressure to the area surrounding the lanced site when 'milking' the site to assist bleeding. Plasters were used to cover the lanced fingers in order to prevent possible infection.

BD Vacutainer® (BD, Plymouth, UK) blood collection tubes (9 mL) containing K₂ EDTA were used to collect samples for GLP-1 and insulin analysis. Approximately 2 mL of blood was discarded before blood was inserted via a syringe (BD Luer Lock, Plymouth, UK) into the blood collection tubes. Tubes were stored on ice prior to sampling and remained on ice before being placed into the centrifuge (within 5 mins of withdrawal). Blood was centrifuged at 3,000 rpm for 15 minutes at 4°C, as per Millipore Merck Enzyme linked immunosorbent assays (ELISA) protocols for human insulin (EZHI-14K) and GLP-1 (EZGLP-

136K, Merck, Darmstadt, Germany). Plasma was carefully removed and stored in labelled 1.5 ml Eppendorfs at -85°C until ELISA analysis was completed.

4.4.1 Hormone Quantification

ELISAs are a highly robust method for quantifying gut hormone concentrations and are frequently used in appetite research (Nilsson et al. 2008; Beck et al. 2009a; Kim et al. 2009b). A sandwich ELISA was chosen for its high specificity and high sensitivity. The sensitivity of the chosen assays, both manufactured by Millipore, were 1 μ U/mL (20 μ L sample size) for insulin and 1.5pM (50 μ L sample size) for total GLP-1.

4.4.2 GLP-1 Standard and Quality Control Preparation

Manufacturer's GLP-1 standards and quality controls (QC) were prepared prior to analysis. Standards and QCs were provided in lyophilized vials, which were reconstituted by adding 0.5 mL de-ionized water. For standard preparations, 200 μ L assay kit buffer was added to five tubes before three-fold serial dilutions were carried out. Pipette tips were changed between dilutions. Standards and QCs were stored in small aliquots at -85°C.

4.4.3 Total GLP-1 ELISA

Standards, QCs and plasma samples were removed from the -85°C freezer (UFV700, Binder© GmbH, Tuttlingen, Germany) on arrival to the laboratory and thawed at room temperature. Once thawed, samples were vortex mixed before being centrifuged for 10 minutes at 3,000 rpm, at 20°C.

All reagents from the Millipore total GLP-1 ELISA kit were equilibrated at room temperature before the assay was set up. The Microtitre Assay Plate (96-well plate) was removed from the sealed foil pouch and wells were labelled appropriately. An 8-channel ELISA plate washer (CAPP wash™ 8, Nordhausen Germany) was used for all plate washing during the assay procedure with horseradish peroxidase wash buffer (1:10 dilution with 900 mL de-ionised water). The 96-well plate was washed three times and tapped

smartly onto absorbent towels. An Eppendorf Multipette E3® (Stevenage, UK) was used to dispense 50 µL of matrix solution to all blank, standards and QC wells. Assay buffer, 50 µL, was added to all blank and sample wells. Duplicate 50 µL GLP-1 standards were then added in ascending concentrations to the appropriate wells of the plate, along with QCs. Sequentially, 50 µL of plasma samples were added in duplicate to the remaining wells. The plate was covered with a plate sealer and incubated at room temperature for 1.5 hours on an orbital microtitre plate shaker, which was set to rotate at a moderate speed of 450 rpm. The 96-well plate was then removed and washed three times with wash buffer and tapped to remove residual buffer. Detection antibody solution was added to all wells (100 µL) using an eight-channel electronic pipette (Eppendorf Xplorer® Stevenage, UK). The 96-well plate was re-sealed and placed back onto the orbital microtitre plate shaker (SSM5 Microtitre Plate Shaker, Stuart, UK) to incubate at room temperature for 1 hour at 450 rpm. Following incubation, the 96-well plate was washed three times and tapped before 100 µL of enzyme solution was added to all wells. The plate was then sealed and placed back onto the orbital shaker for 30 minutes. After the incubation period, the plate was washed three times and tapped before 100 µL of substrate solution was added to all wells. The plate was sealed and placed back onto the orbital shaker for 5 - 20 minutes, which was time dependent on the speed of the colour change. A blue colour developed in wells of the GLP-1 standards with intensity that was proportional to the increasing concentrations of total GLP-1. Colour development was monitored visually to optimize incubation time. The plate was removed from the plate shaker after approximately 12 minutes of incubation and 100 µL of stop solution was added to all wells. Acidification resulted in a colour change from blue to yellow. The plate was gently shaken by hand to remove air bubbles and to ensure complete mixing of the solution in all wells. The 96-well plate was then placed in a Microtitre plate reader (BioTek Powerwave 200, UK). Absorbance was read at 450 nm within five minutes of the stop solution being added. A quantitative curve fitting program for immunoassays (MasterPlex 2010, MiraiBio Group of Hitachi Solutions, USA), which used a 5 Parameter Logistic model equation, was used to fit a standard curve and determine samples for GLP-1 concentrations. Values, including means, SD and %CV were copied to a Microsoft Excel file. Samples which had a %CV of >15 were re-assayed.

4.4.4 Insulin ELISA

A similar protocol was carried out to quantify insulin. The protocol followed a typical sandwich ELISA, which quantified antigens between two layers of antibodies. As with the GLP-1 96-well plate, the ELISA plate for insulin quantification was pre-coated with capture antibodies by the manufacturer.

All reagents from the Millipore Insulin ELISA kit were equilibrated at room temperature before use. Insulin standards and QCs did not require prior preparation. Samples were thawed and prepared for analysis as previous. The 96-well plate was removed and labelled appropriately before being washed once and tapped on absorbent paper. Assay buffer was then added to all blank and sample wells (20 μ L), along with 20 μ L of matrix solution to all blank, standard and sample wells. Duplicate 20 μ L insulin standards, QCs and plasma samples were added to the appropriate wells. An Eppendorf Multipette E3® was used to add 20 μ L of detection antibody to all wells before being incubated for 1 hour on an orbital plate shaker at room temperature. The 96-well plate was then washed three times with wash buffer and tapped to remove residual buffer before 100 μ L of enzyme solution was added to all wells. The 96-well plate was left to incubate as before for 30 minutes. The 96-well plate was washed five times with wash buffer and tapped before 100 μ L of substrate solution was pipetted into all wells. As before, the researcher monitored the colour change. The 96-well plate was removed from the plate shaker after approximately 10 minutes of incubation and 100 μ L of stop solution was added to all wells. The 96-well plate was read in the Microtitre plate reader (Biotek Powerwave 200) at 450 nm and concentrations of insulin were quantified using MasterPlex 2010. Any samples which had a %CV of >15 were re-assayed.

4.5 Anthropometry

4.5.1 Waist Circumference

Subjects assumed a relaxed standing position with their arms folded across their thorax with their feet approximately 30 cm apart. The measurement was taken at the narrowest point between the lower costal border (10th rib) and iliac crest. If there was no obvious narrowing, the measurement was taken at the midpoint between these two landmarks. The subject was instructed to lower their arms to a relaxed position, with the tape being readjusted if required. The researcher followed the normal breathing pattern of the subject and took the measurement at the end of their normal expiration. All WC measurements were taken in accordance to ISAK (2001) protocol, which is the ‘gold standard’ for measuring abdominal body girth.

4.5.2 Height, Weight and Body Mass Index

Subjects were required to wear light clothing and no shoes. Body weight was measured to the nearest 0.1 kg using an automated calibrated electronic scale (Salter© 90185S SV3R, Kent, UK). Before subjects were asked to stand on the scales they removed all items from their pockets, if necessary. Standing height was measured without shoes to the nearest 0.1 cm using a fixed wall stadiometer (Seca 206, Birmingham, UK). Subjects stood erect on the floor with their back to the stadiometer, their arms hanging by their sides with their weight evenly distributed on both feet. The horizontal bar was lowered to make contact with the crown of the subject’s head with sufficient pressure to compress the hair. The researcher ensured the subject’s head was positioned in the Frankfort Horizontal Plane position. The Frankfort Plane is an imaginary line passing through the external ear canal and across the top of the lower bone of the eye socket, immediately under the eye (ISAK 2001). Accessories worn in the hair were removed from the subject in order to obtain an accurate measurement. Weight was coupled with height to calculate BMI as per the following equation (WHO 2000): $BMI = kg / m^2$.

4.6 Rheometry

4.6.1 Viscosity Profiling

Viscosity is the measure of the internal friction of a fluid. This friction becomes apparent when a layer of fluid is made to move in relation to another layer. The greater the friction, the greater the amount of force required to cause this movement, which is called shear. Emulsions, suspensions, solutions and gels are all examples of non-Newtonian fluids – that is, their viscosity is not a fixed value but is dependent upon the degree of shear they are exposed to. By far the most common form of non-Newtonian behaviour is shear-thinning, where viscosity decreases with increasing applied shear rate (Tian et al. 2018).

4.6.2 Rotational Rheometer

Rotational viscometers use the idea that the force required to turn an object in a fluid can indicate the viscosity of that fluid. Continuous measurements at a set of different shear strain-rates for one sample can be performed to analyze shear dependent behaviour, and as a result can test both Newtonian and non-Newtonian fluids. Using a rotational rheometer is advantageous for β -glucan containing samples as MW determines physiochemical properties. At a low concentration ($<0.2\%$) β -glucan solutions behave like Newtonian solutions, i.e., an increasing shear-rate does not affect viscosity, whereas above 0.2% high MW β -glucan molecules entangle and form viscous and pseudoplastic solutions (Doublier and Wood 1995; Anttila et al. 2004).

Rotational rheometers equipped with parallel plates are widely used to measure rheological properties of foods (Mezger 2011). Most commonly the upper plate is rotatable and the bottom plate is stationary. The diameter of the bottom plate is larger than the diameter of the upper plate.

The height of the sample should be less than the upper plate radius, thus the gap size can impact the results gained from the rheometer. For example, a large gap size between the two plates can result in edge failure for visco-elastic samples or inhomogeneous deformation for paste-like samples. **Figure 10** shows appropriate positioning and filling of samples for viscosity measurement using a rotational rheometer.

A constant shear rheometer, Malvern Bohin C-VOR 150 (Malvern, UK), with a 4 °C, 40 mm diameter cone and plate geometry was used for all rheometry measurements.

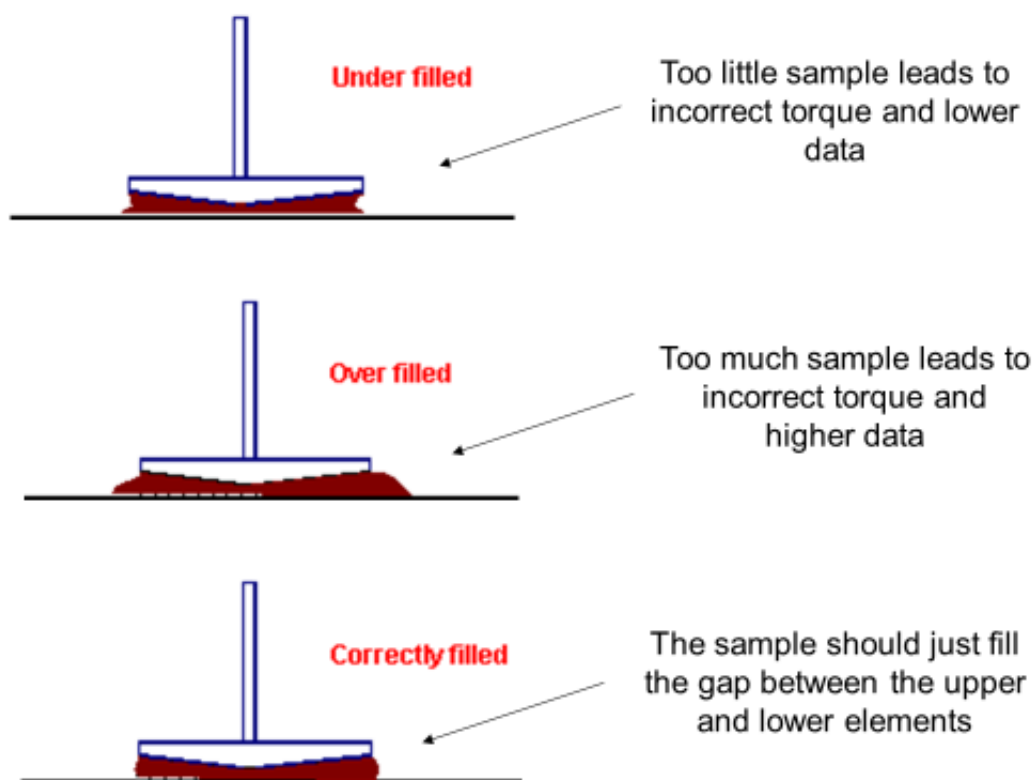


Figure 10. Appropriate sample positioning in the rheometer parallel plate measuring system. Figure prepared using information taken from (Mezger 2011).

4.6.3 Sample preparation

Inaccurate sample preparation can negatively influence the results of rheological properties if care is not given to the correct procedure. During testing it was essential that the sample maintained contact with the upper and lower plates. The samples should not be over-compressed to avoid pre-stressing effects and liquid migration. Trial runs were performed in order to determine adequate volumes of each sample, thus uniform samples were tested for all experiments.

A measurement of viscosity for the entire food matrix (as it would be under gastric & small intestinal conditions) was not possible for technical reasons, i.e., Rice Krispies plus yoghurt, plus milk would not allow for a homogeneous sample to be measured accurately with our method. Nevertheless, with reference to the recent review by Poutanen et al. (2017), recognition of physiochemical properties of fibre containing foods is important and even minimal basic viscosity measurements should be acknowledged in acute appetite studies to allow for possible links to be made with physiological satiating mechanisms.

In order to determine the viscosity of the test breakfasts, breakfasts were prepared as eaten by subjects. The following samples were prepared, i) 90 g Greek-style yoghurt, ii) 80 g Greek-style yoghurt and 7.3 g OatWell™ oat bran, iii) 150 mL semi-skimmed milk and iv) 150 mL semi-skimmed milk and 7.3 g OatWell™ oat bran.

4.6.4 Steady shear viscosity

Samples were characterized for their steady shear viscosity. Steady shear viscosity was measured by increasing the amount of stress to all samples. Parameters of this test varied depending on the viscosity of each sample to create a curve for shear stress rates. Measurements were carried out at 37°C to mimic stomach temperature and at shear rates ranging from 0.5×10^{-1} to $1.0 \times 10^2 \text{ s}^{-1}$. A shear rate of 50 s^{-1} was used to represent gastric conditions.

4.7 Statistical analysis

The primary outcome of this study was *ad libitum* energy intakes (kcal), thus the study recruited a minimum of 36 subjects to detect changes in this outcome measure.

Data for VAS ratings (hunger, fullness, desire to eat, satiety and prospective food consumption), energy intakes and results from blood analysis were initially inputted into a Microsoft excel spreadsheet before being transferred to SPSS software (version 23.0; Chicago, IL, USA) to perform data distribution tests. Normality of all data were tested using Shapiro–Wilk statistic. Differences in energy intake between the

two treatments were assessed using Student's paired samples t-test. Total AUCs for subjective appetite ratings, blood glucose and hormones were calculated using the trapezoidal method. Subjective appetite ratings were analysed using ANCOVA with baseline values used as a co-variate (Blundell et al. 2010). Time x treatment effects for blood glucose and hormones were identified using a two-factor analysis of variance (2 factor-ANOVA) with time and treatment (breakfast) as factors. *Post hoc* comparisons, adjusted for multiple comparisons by Bonferroni's correction, were performed where ANOVAs revealed significant effects to identify differences between treatments across timepoints. Paired t-tests were performed to compare viscosity data of milk and Greek-style yoghurt with and without OatWell™ oat bran at 50s⁻¹. All tests were two tailed and significance was set at $p < 0.05$. All values are presented as means \pm standard error of the mean (SEM).

Chapter 5

5.0 Effects of oat β -glucan consumption at breakfast on *ad libitum* eating, appetite, glycaemia, insulinaemia and GLP-1 concentrations in healthy subjects (Study A): Results

5.1.0 Recruitment

The flow of participants through the study, from recruitment to study completion and analysis, can be seen in **Figure 11**. One hundred and forty-two individuals responded to the recruitment material and contacted the researcher to express interest in study participation. Of the 142 responses, 48 individuals attended the screening session at Queen Margaret University. Reasons for not attending the screening session were as follows: no response, $n=27$; time constraints, $n=39$; GI intolerances, $n=10$; smokers, $n=7$, other $n=11$. Forty-three individuals met the inclusion criteria and enrolled onto the study. Five individuals were excluded from participation; one individual had a BMI $> 29.9 \text{ kg/m}^2$, one subject had a fasted blood glucose measurement $> 5.6 \text{ mmol/L}$ and took antihypertensive medication, two subjects had a BMI $< 20 \text{ kg/m}^2$, and one subject had a BMI $< 20 \text{ kg/m}^2$ and was perimenopausal. Four subjects withdrew from the study before attending the first study morning; three subjects had work commitments and one subject did not respond to schedule study mornings. A total of 36 subjects completed both study mornings, with three subject dropouts during the testing period. One subject felt uncomfortable with the IV cannula and did not wish to continue with the study, and two subjects did not want to consume the *ad libitum* lunch.

Two subjects did not consume the test breakfasts and one subject had an elevated fasted blood glucose measurement on arrival to one study morning. As a result, three subjects were excluded from the final analysis as they did not adhere to the study protocol. All subjects completed the study within a month of attending the first study morning.

Complete blood samples were unable to be withdrawn from all subjects. On a few occasions, the 90 minute blood sample was missed due to the IV cannula losing patency. Only subjects with complete plasma

samples were analysed for total GLP-1 and insulin (n=21). There was no missing data for blood glucose (n=33).

Following analysis of VAS, it was evident that two subjects (Subject 201, Subject 204) did not fully understand the appropriate completion of the VAS. Their responses were erratic in nature, as shown in **Appendix 6**, therefore removed from subjective appetite rating analysis.

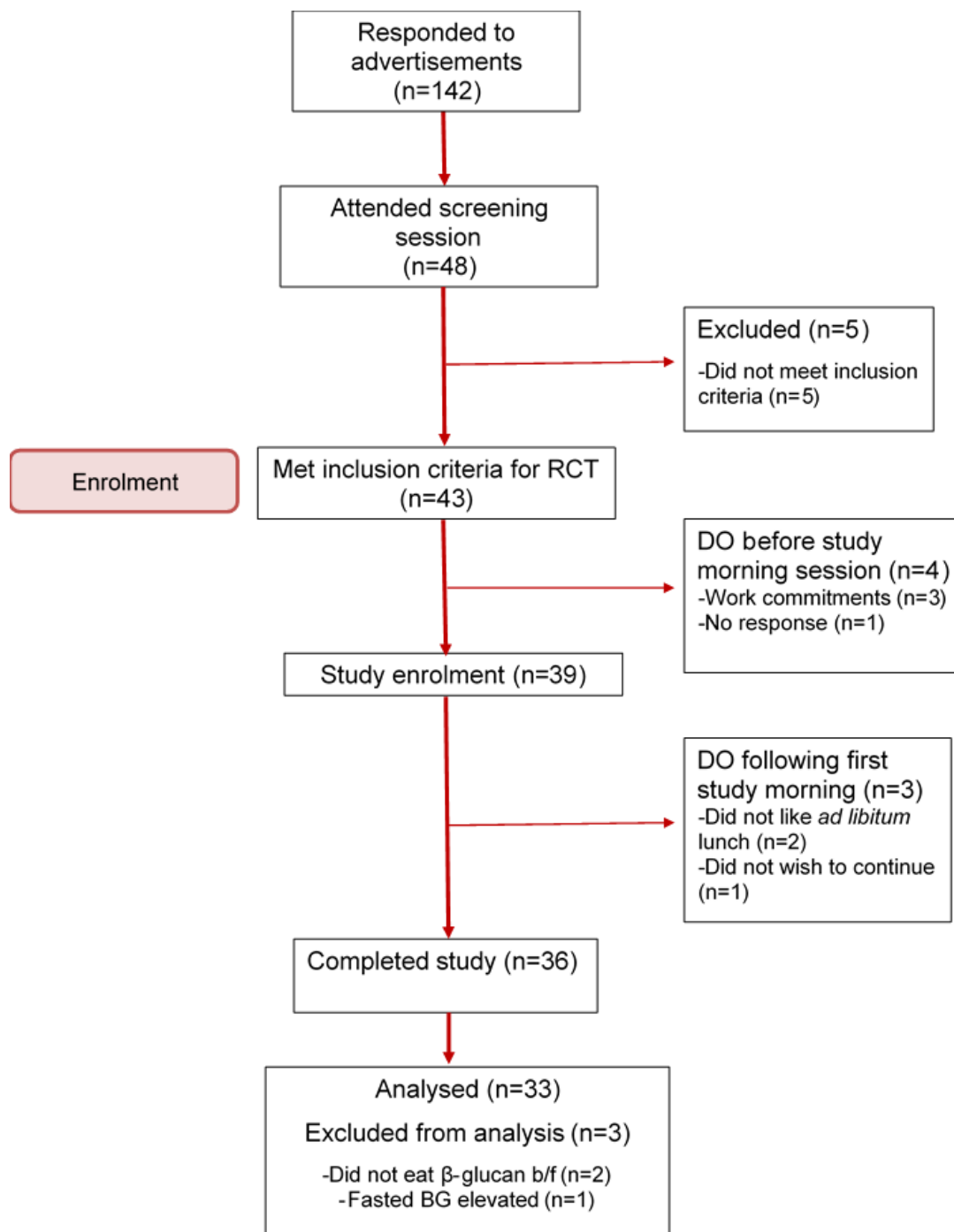


Figure 11. Overview of subjects from recruitment to study completion. *b/f* breakfast; *BG* blood glucose; *DO* drop out; *RCT* randomised controlled trial

5.1.1 Subjects characteristics

All subjects were healthy and reported to habitually consume breakfast every day. None of the subjects were smokers, or were under a calorie-restricted diet. All subjects reported to be weight stable. **Table 9** summarises subject characteristics from information collated during screening sessions.

Table 9. Subject characteristics from screening visit

Parameter	mean
Sex	22 females / 11 males
Age (years)	27 ± 1
Height (m)	1.70 ± 0.1
Weight (kg)	68.1 ± 2.0
BMI (kg/m ²)	23.5 ± 0.4
WC (cm)	78.0 ± 1.5
Physical activity (mins/week)	873 ± 118
Restraint eating score	2.29 ± 0.12
Fasted blood glucose (mmol/L)	4.7 ± 0.1
Haemoglobin (g/L)	133 ± 2
Breathing rate (bpm)	14 ± 0.3
Temperature (°C)	36.6 ± 0.1
SBP (mmHg)	133 ± 2
DBP (mmHg)	72 ± 2
Heart rate (bpm)	69 ± 2
Data are means \pm SEM, n=33	

5.1.2 Adverse effects

No subjects withdrew from the study due to adverse effects from breakfast consumption. Both breakfasts were generally well tolerated, with a few minor GI side effects reported. No GI changes were reported as severe. **Table 10** details the severity and frequency of reported GI side effects reported during the study period.

Within 24 hours of consuming the β -glucan test breakfast, 10 subjects reported flatulence, six subjects reported bloating and five subjects reported changes in their stools. There were fewer GI disruptions following control breakfast consumption. Three subjects reported flatulence, four subjects reported bloating and changes in their stools.

5.1.3 Subsequent day

Fewer GI side effects were reported on the day following the study session, i.e., 24 – 48 hours following test breakfast consumption. Four subjects reported flatulence and bloating following the β -glucan test breakfast. A total of six subjects reported changes in their stool. One subject reported flatulence the day after the control breakfast was consumed. Three subjects reported bloating and two reported changes in stools (**Table 10**).

Table 10. Gastrointestinal side effects reported in food diaries following study sessions for both control and intervention breakfasts.

	Flatulence			Bloating			Changes in stool		
	mild	moderate	a lot	mild	moderate	severe	mild	moderate	severe
Test day									
Control	3			4			3	1	
β-glucan	7	3		3	3		2	3	
Subsequent day									
Control	1			3			2		
β-glucan	4			3	1		3	3	

Data collated from all subjects who returned food diaries, n=32

5.2 Energy intake

5.2.1 Day before study

Energy intakes for all subjects were not significantly different between treatments the day prior to study mornings, with mean values of 1845 ± 95 kcal consumed the day prior to the control treatment and 1851 ± 115 kcal consumed the day prior to β-glucan treatment ($t(31)=-0.43$, $p=0.94$).

5.2.2 *Ad libitum*

There was no effect of treatment on *ad libitum* eating. Total intakes at the test meal were 681 ± 46 kcal and 267 ± 18 g with the breakfast containing oat β-glucan and 704 ± 51 kcal and 275 ± 20 g with the control breakfast ($t(32)=0.875$, $p=0.388$ and $t(32)=0.846$, $p=0.404$, respectively).

There were no differences between time spent to consume the test breakfasts, 11 ± 4 minutes and 11 ± 5 minutes, $t(32)=49$, $p=0.99$. There was also no difference in water intake at the *ad libitum* meal ($t(32)=-0.32$, $p=0.751$, **Table 11**).

5.2.3 Subsequent energy intake

The oat β -glucan breakfast did not detectably affect subjects' energy intake for the remainder of the study day when compared with the control breakfast ($t(31)=-1.70$, $p=0.099$, **Table 11**).

With regards to the day following the study, there were no significant changes in caloric intakes following the control (1858 ± 115 kcal) and β -glucan testing session (1924 ± 124 kcal), ($t(31)=-0.5$, $p=0.62$).

Table 11. Energy intakes of subjects before and after consumption of test breakfasts

	Control	β -glucan	p value
Food intake <i>ad libitum</i> test meal (kcal)	704 ± 51	681 ± 46	0.388
Food quantity at <i>ad libitum</i> test meal (g)	275 ± 20	267 ± 18	0.404
Water intake <i>ad libitum</i> test meal (mL)	213.1 ± 11.3	218.2 ± 15.1	0.751
Food intake remainder of study day (kcal)	886 ± 91	1094 ± 120	0.099

Data are from $n=33$ subjects for food and water intake at *ad libitum* meal. One subject failed to return their food record for the remainder of both study days, $n=32$. Data are means \pm SEM

5.3 Subjective appetite ratings

There was a significant effect on total AUC of fullness ratings after controlling for baseline AUC, ($F[1,60]=2.98$, $p=0.048$). Total AUC for fullness following oat β -glucan and control breakfast were 7563 ± 428 mm x min and 6505 ± 453 mm x min, respectively (**Figure 12, panel A**).

There was also a significant effect of the oat β -glucan breakfast on total AUC of satiety ratings after controlling for baseline AUC, ($F[1,60]=3.07$, $p=0.034$). Total AUC for satiety following oat β -glucan and control breakfast were 7604 ± 459 mm x min and 6516 ± 427 mm x min, respectively (**Figure 12, panel B**).

No effects of oat β -glucan on hunger (**Figure 13, panel A**; $p=0.133$), desire to eat (**Figure 13, panel B**; $p=0.098$) or prospective food consumption (**Figure 13, panel C**; $p=0.213$) were reported.

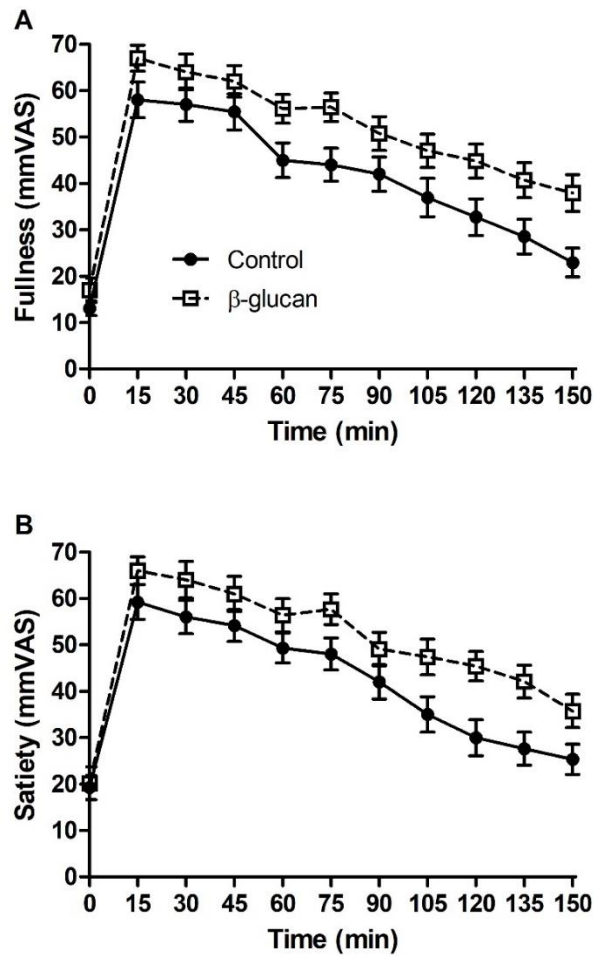


Figure 12. Visual analogue scales (VAS) for subjective ratings of fullness (A) and satiety (B) during the 150-min postprandial period following control (●) and β -glucan (□) breakfast consumption. Data were analysed by ANCOVA using baseline value as a co-variate. Data are means \pm SEMs ($n=31$, two subjects were excluded from data analysis as they misunderstood the VAS questionnaires).

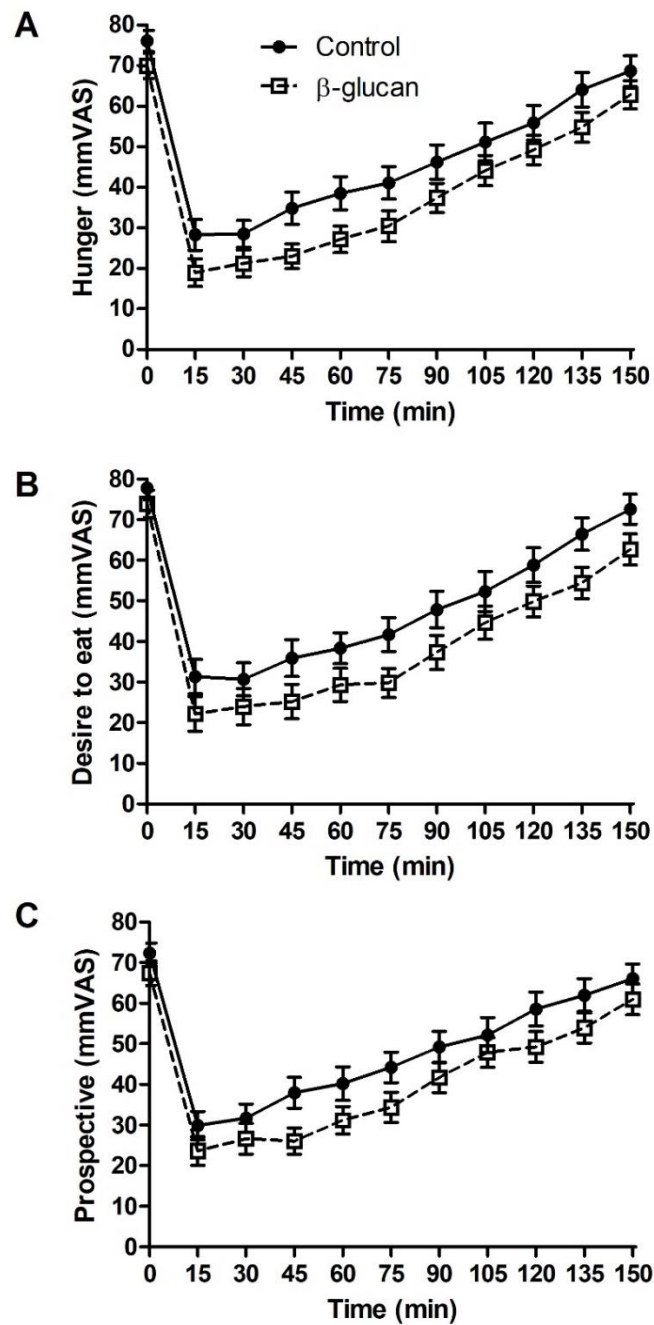


Figure 13. Visual analogue scales (VAS) for subjective ratings of hunger (A) and desire to eat (B) and prospective food consumption (C) during the 150-min postprandial period following control (●) and β-glucan (□) breakfast consumption. Data are means ± SEMs (n=31).

5.4 Plasma hormone and glucose concentrations

Figure 14 presents glucose, plasma insulin and plasma total GLP-1 responses over the 90-minute postprandial period.

There were no differences in baseline (fasting) values between study days for total GLP-1 ($t(20)=-1.76$, $p=0.09$), blood glucose ($t(32)=0.29$, $p=0.771$), or plasma insulin ($t(20)=-1.40$, $p=0.176$, **Figure 14**).

5.4.1 GLP-1

For plasma GLP-1, Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $X^2(5) = 9.59$, $p=0.03$, and therefore, a Greenhouse Geisser correction was used. There was a treatment x time interaction ($F[2.3,45.3]=6.62$, $p=0.002$) for GLP-1. Plasma GLP-1 concentrations were significantly reduced at 90 min after the oat β -glucan breakfast when compared with the control breakfast (22 ± 9 pmol/L vs. 17 ± 9 pmol/L, $t(20)=2.50$, $p=0.021$, **Figure 14, panel A**).

There was no significant difference for GLP-1 AUCs between treatments ($t(20)=0.59$, $p=0.56$, **Table 12**).

Only subjects with complete data sets were included in the analysis for plasma GLP-1 (full data, $n=21$).

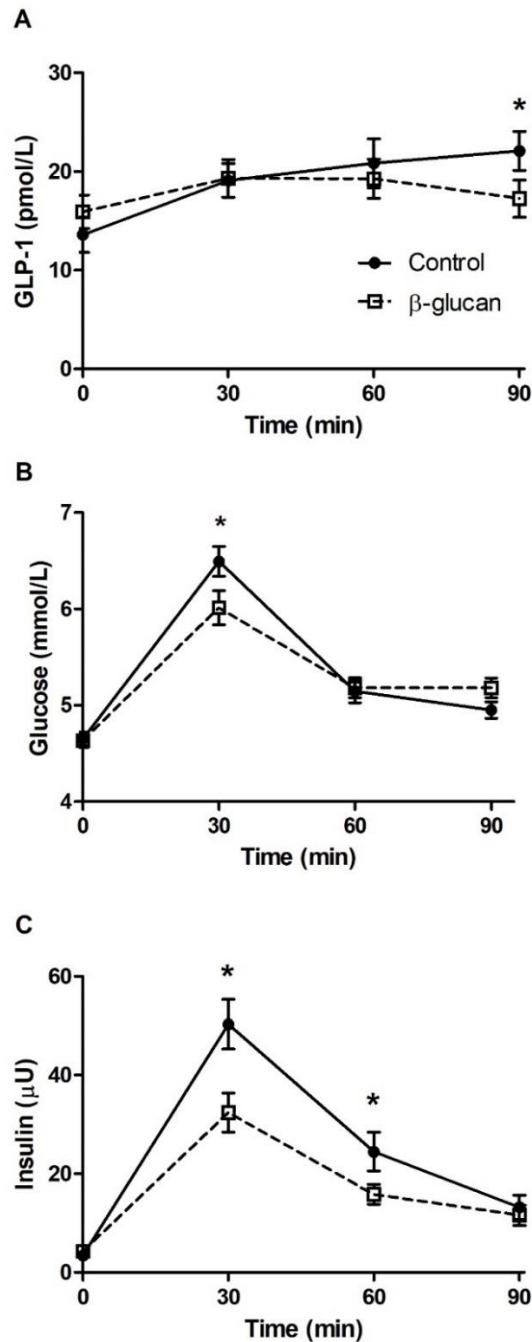


Figure 14. Plasma glucagon-like peptide-1 (A), blood glucose (B), and plasma insulin (C) concentrations during the 90-min postprandial period following control and β -glucan breakfast consumption. Data were analysed with two-factor ANOVA, with treatment and time as factors. In case of significant differences, *post hoc* comparisons, adjusted for multiple comparisons by Bonferroni correction, were performed to determine significant differences between the control (●) and β -glucan (□) breakfasts. * $p < 0.05$. Data are means \pm SEMs (A, $n = 33$; B and C, $n = 21$, 12 subjects were excluded from the analysis due to incomplete data sets).

5.4.2 Glucose

For blood glucose, Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $X^2(5) = 16.78$, $p=0.005$, and therefore, a Greenhouse Geisser correction was used. There was a treatment x time interaction ($F[2.3,72.3]=49.13$, $p<0.001$) for blood glucose. Blood glucose was significantly lower at 30 min after the oat β -glucan breakfast when compared with control (6.0 ± 1.0 mmol/L vs. 6.5 ± 0.9 mmol/L, ($t(32)=2.81$, $p=0.008$)) (**Figure 14, panel B**).

There was no significant difference for blood glucose AUCs between treatments ($t(32)=1.21$, $p=0.235$, **Table 12**).

5.4.3 Insulin

For plasma insulin, Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $X^2(5) = 12.2$, $p=0.033$, and therefore a Greenhouse Geisser correction was used. There was a treatment x time interaction ($F[2.08,44.1]=56.98$, $p<0.001$) for plasma insulin. Plasma insulin was significantly lower at 30 and 60 min after the oat β -glucan breakfast when compared with control (32.4 ± 18 μ U vs. 50.3 ± 23.1 μ U, $t(20)=3.63$, $p=0.002$) and 15.8 ± 9.2 μ U vs. 24.4 ± 18 μ U, ($t(20)=2.62$, $p=0.017$), respectively (**Figure 14, panel C**).

The AUC for insulin over the 90 min period was also significantly lower following the oat β -glucan breakfast when compared with the control breakfast ($t(20)=3.99$, $p=0.001$, **Table 12**).

Only subjects with complete data sets were included in the analysis for plasma insulin (full data, $n=21$).

Table 12. Area under the curves (AUC) for blood glucose and plasma insulin and total GLP-1 concentrations following control and β -glucan breakfasts

	Control	β -glucan	p-value
Glucose AUC ($\text{mmol} \times \text{L}^{-1} \times \text{min}$)	498.2 ± 47.3	483.0 ± 49.5	0.235
Insulin AUC ($\mu\text{U} \times \text{mL}^{-1} \times \text{min}$)	2491.0 ± 1211	1682.2 ± 902.4	0.001
Total GLP-1 AUC ($\text{pmol} \times \text{L}^{-1} \times \text{min}$)	1732.7 ± 713	1654.7 ± 706.8	0.560

Data are from n=33 subjects for blood glucose and n=21 for plasma insulin and total GLP-1. AUC based on 0-90 min data. Data are means \pm SEM

5.5 Sensory feedback

5.5.1 Test Breakfasts

There were no significant differences in smell or aftertaste when control and β -glucan breakfasts were compared ($p=0.06$, $p=0.71$, respectively). Visual appeal, taste and palatability of the β -glucan breakfast were scored significantly lower compared to the control breakfast ($p<0.001$). **Figure 15** shows subjects' perceptions of each test breakfast in relation to the sensory characteristics scored.

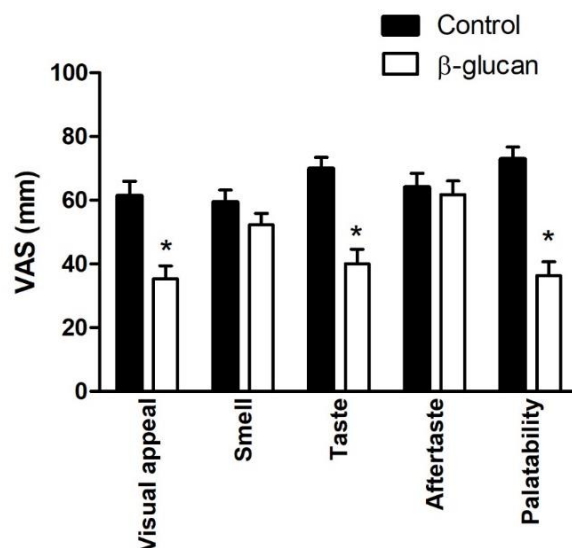


Figure 15. Subjects' perceptions of each test breakfast. * $p < 0.001$ following paired samples t-tests (two-tailed) between control and β -glucan breakfast scores. Data are means \pm SEM

5.5.2 *Ad libitum* lunch

The ham sandwiches consumed at the *ad libitum* lunch were generally well accepted. There were no statistical differences between sensory perceptions for all characteristics scored between visits ($p > 0.05$), therefore the ham sandwiches were similarly liked during each study session. Out of a possible score of 100 mm (0 'bad' vs. 100 'good'), the mean score from both test lunches for visual appeal was 72.1 ± 20.5 mm, smell 74.3 ± 17.7 mm, taste 77.2 ± 19.2 mm, aftertaste 80.8 ± 17.3 mm and palatability 81.8 ± 17.3 mm.

5.6 Viscosity of test breakfasts

The viscosity of the different components of the breakfast containing oat β -glucan was considerably greater than that of the control breakfast, with significant differences in viscosity seen at a rate of 50 s^{-1} ($p < 0.001$), a shear rate representative of gastric conditions. **Figure 16** shows viscosity developments in response to oat β -glucan.

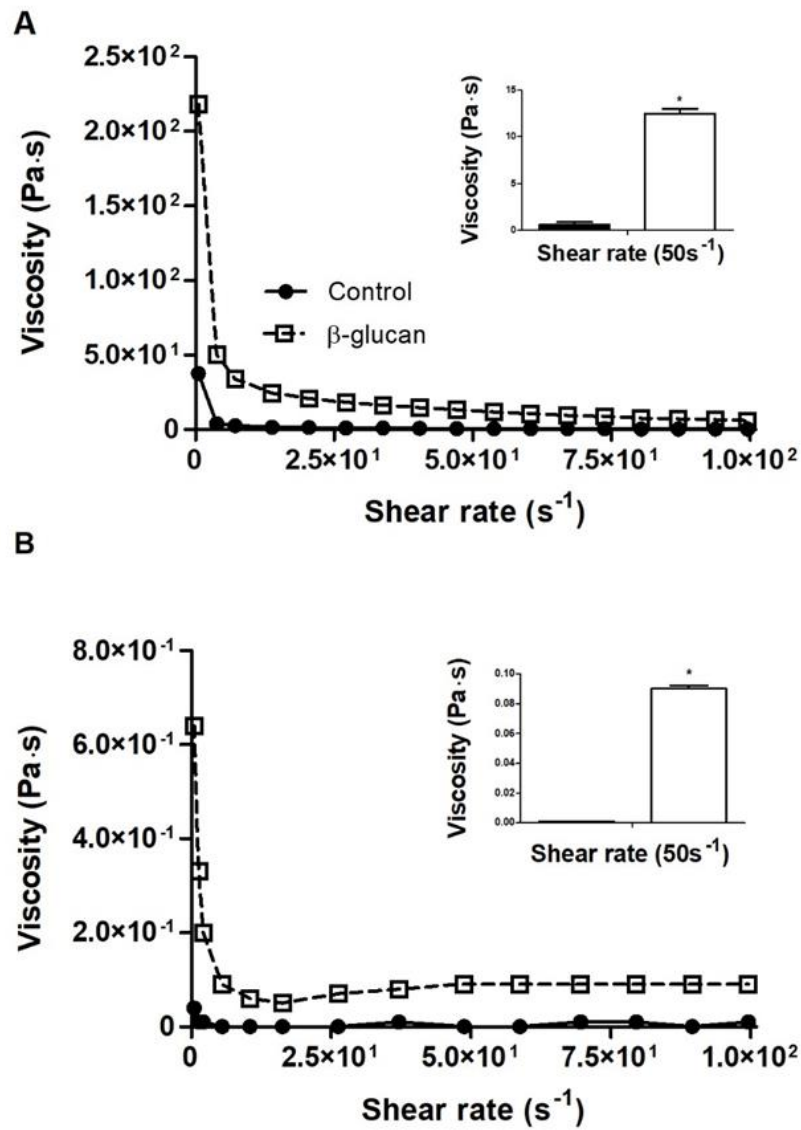


Figure 16. Viscosity of both, the yoghurt (A) and milk (B) component of the test meal containing high-molecular weight oat β -glucan (\square) or control (\bullet) across different shear rates ranging from 0.5×10^{-1} to $1.0 \times 10^2 s^{-1}$. Inserts depict a shear rate of $50 s^{-1}$, representative of gastric conditions. * $p < 0.05$

Chapter 6

6.0 Effects of oat β -glucan consumption at breakfast on *ad libitum* eating, appetite, glycaemia, insulinaemia and GLP-1 concentrations in healthy subjects (Study A): Discussion

The evidence for cereal β -glucans to lower appetite and *ad libitum* eating is contradictory and the underpinning mechanisms, particularly GI satiation peptide secretion, unclear. The effect of high MW oat β -glucan incorporated into a breakfast meal on *ad libitum* eating, subjective appetite, plasma GLP-1 and insulin as well as blood glucose concentrations in 33 healthy subjects was investigated in the current study. Based on previous studies (Beck et al. 2009a; Juvonen et al. 2009; Rebello et al. 2016a), it was hypothesized that oat β -glucan would increase fullness and/or satiety and reduce *ad libitum* eating associated with changes in plasma GLP-1 and a reduction in blood glucose and plasma insulin. It was found that subjects were more satiated and fuller after consuming the oat β -glucan breakfast when compared to the control; however, this did not translate into a reduction in food intake neither during the *ad libitum* test meal, nor for the remainder of the day or subsequent day. There was also no increase in plasma GLP-1; however in contrast, this study found a small but significant decrease in GLP-1 90 minutes after the breakfast with oat β -glucan was consumed. In line with the literature, a significant reduction in postprandial blood glucose and plasma insulin was observed (Wanders et al. 2011; Tosh 2013).

6.1 Energy Intake and Appetite

6.1.1 Eating-inhibitory effect and subjective ratings of appetite

The potency of oat and barley β -glucan to modulate appetite has been reported in several studies, although the effect seems to vary depending on the study design, subject characteristics (e.g. BMI, sex), and the dose and MW of β -glucan consumed. Doses of cereal β -glucan have been reported to range from 2.2 g to 9.4 g, with varying or unreported MWs (Beck et al. 2009a; Lyly et al. 2009; Vitaglione et al. 2009; Willis et al. 2009; Clegg and Thondre 2014). Additionally, the manufacturing process such as baking, cooking or

extrusion (Hu et al. 2010; Ames et al. 2015b) and other test food characteristics have also varied considerably (El Khoury et al. 2012; Rebello et al. 2014). Fullness was increased in 14 healthy overweight subjects following a 3.82 g dose of β -glucan oat bran cereal with a high MW of 1.378×10^6 g/mol consumed at breakfast in a study conducted by Beck et al. (2009a), and similarly by Pentikäinen et al. (2014) following 4 g of high MW β -glucan incorporated into biscuits and juice consumed at breakfast by normal weight subjects. In contrast, Beck et al (2009a) reported no effect on subjective appetite ratings following a 5.65 g high MW β -glucan containing breakfast in overweight subjects, and similarly by Juvonen et al. (2011), there was no appetite effect in normal weight subjects after consumption of 5.1 g high MW β -glucan containing puddings.

The findings of the current study are in line with several studies and suggest that oat β -glucan beneficially modulates appetite by increasing fullness and satiety. Manufacturing processes such as baking, cooking or extrusion (Hu et al. 2010; Ames et al. 2015b) and other test food characteristics including food format may also affect the satiating capacity (El Khoury et al. 2012; Rebello et al. 2014). The physicochemical properties of the matrix in which the fibre is delivered in combination with the gut environment play a critical role in determining the hydration or swelling and water-retention capacity of the fibre (Rebello et al. 2016b). In this study, β -glucan was consumed in a semi-solid food matrix, with yoghurt and cereal with milk used as the vehicle to deliver β -glucan. Other studies that have used a semi-solid food matrix also reported increased satiety and fullness with test meals containing 1.6 to 4 g oat β -glucan (Rebello et al. 2014; Geliebter et al. 2015; Rebello et al. 2016a). However, Juvonen and colleagues (2011) found no effect on subjective appetite following a semolina-based pudding that contained 5.1 g oat β -glucan, suggesting that the food matrix alone does not determine oat β -glucan's satiating capacity. However, more research is thus warranted to better understand how the satiating capacity of β -glucan depends on experimental paradigms, population characteristics and fibre/food format features. Studies should be well controlled, particularly with regards to the target population to reduce inter-individual variability in, for example, GI transit, which in many cases directly influences the physiologic effects of fibre (Collins et al. 1983; Poutanen et al. 2017). This includes population characteristics such as body weight, age, sex, or habitual

diet, which all can influence GI transit (Wang et al. 2015). Ultimately, a clear hypothesis-led primary outcome and appropriate statistical power considerations are relevant to ensuring a reliable effect size estimate and understanding of results.

In the current study, the beneficial effect on subjective appetite did not translate into a decrease in food intake at the *ad libitum* test meal, which is corroborated by literature that have reported similar dissociations following oat β -glucan consumption (Beck et al. 2009a; Clark and Slavin 2013). It is important to note that although appetite VAS are generally sensitive to experimental manipulations and are reproducible, they have failed to predict meal size under a number of conditions (Stubbs et al. 2007; Flint et al. 2000; Beck et al. 2009a). The magnitude of differences in self-reported VAS which precede *ad libitum* eating were investigated recently by Sadoul et al. (2014). Based on a large number of studies that used a wide range of nutrient preloads, retrospective analysis of 23 randomized controlled appetite trials reported that a significant difference in energy intake at lunch was likely to be achieved if the difference in satiety (intervention vs. control) immediately before the *ad libitum* meal initiation was at least 15–25 mm on a 100 mm scale (Sadoul et al. 2014). In addition, self-reported hunger prior to a meal added significantly, but only modestly, to other variables predicting subsequent food intake. Sadoul et al. (2014) suggest that the minimum change in VAS needed varied with the initial hunger value. When very high initial hunger levels were apparent, a larger intervention impact was needed before that was translated into a change in energy intake. With reference to the findings of the current acute study, relatively high hunger ratings were recorded by subjects at baseline and there was no difference between hunger ratings immediately before *ad libitum* meal initiation (**Figure 13, Chapter 5**). With regards to all other subjective appetite measures, there were no mean differences between breakfasts of more than 15 mm before *ad libitum* meal onset, which may possibly explain why changes in energy intake were not seen. Furthermore, Sadoul and colleagues (2014) report that energy intake behaviour appeared to be more sensitive to small changes in motivation state, rather than initial high hunger levels. This suggests that satiety interventions may potentially be more effective within weight management programmes that advocate eating regular meals and snacks, thus

avoiding extreme hunger levels. Consumption of cereal β -glucan between meals in conjunction with hypocaloric diets is an avenue for future research.

Whether a different inter-meal interval or a higher dose of oat β -glucan may have resulted in significant eating effects should be investigated more comprehensively, for example by using varying time intervals and doses in the same study. Because satiation depends on both gastric and intestinal nutrient stimulation, and their interactions (Steinert et al. 2017), an optimal dose and timing between preload and *ad libitum* test meal is likely crucial to detect an eating-inhibitory effect. Attention should also focus on identifying an appropriate inter-meal interval in relation to the physiochemical characteristics of β -glucan. Zaremba et al. (2017) suggest that inter-meal intervals of 4 hours or more may be more suitable for fermentable fibres that are suggested to influence appetite processes via the production of short-chain fatty acids (SCFA) produced as a result of colonic microbial fermentation and the subsequent release of GLP-1 and PYY (Chambers et al. 2015; Noak et al. 2015). Perhaps the best method may be to have participants select the time of their next meal. This approach has been rarely followed and could be performed in a free-living setting. Determination of the onset latency of the next meal when freely requested by subjects would allow subjects to avoid high hunger levels and allow consideration of motivational state to influence eating as per the suggestion by Sadoul and colleagues (2014).

Several lines of evidence support the hypothesis that increased gastric volume contributes to satiation (Steinert et al. 2017). Viscous fibres absorb large quantities of water and most studies link ingestion of viscous dietary fibres to delayed GE (Bergmann et al. 1992; Benini et al. 1995; Marciani et al. 2000; de Graaf et al. 2004), which will increase gastric-volume signals. Bergmann et al. (1992), for example, found sensations of satiety and hunger highly correlated with GER following consumption of viscous psyllium fibre ($r^2=0.978$, $p<0.001$). Gastric emptying was not measured in the current acute study, which reflects a limitation, however, there are a few studies that report a slowing of GE with cereal β -glucans under similar conditions (Juntunen et al. 2002; Yu et al. 2014; Geliebter et al. 2015).

Solid foods are known to increase satiety and decrease hunger more effectively than liquid ones (Chambers et al. 2015). Thus, the larger satiating effect of solid food *per se* may mask the satiating potential of β -glucans. For example, chewing of solid food could provide a satiety signal not triggered by swallowing liquids containing fibre (Haber et al. 1977). Ingestion of solids and liquids at approximately the same time has been reported to affect the rate of GE and perceived satiety (Horowitz et al. 1989). When a soup was ingested immediately before a sandwich, GE was affected, but when the soup was ingested 20 minutes before the sandwich, there was no effect of the soup on GE observed in two studies by Spiegel and colleagues (Spiegel et al. 1994; Spiegel et al. 1997). These studies suggest that combining liquid and solid foods in a single meal can affect both fullness and GE. The current study contained a mixed solid and liquid meal containing β -glucan, which may explain why increased satiety and fullness were reported. This is also corroborated by Pentikäinen et al. (2014), who report that biscuits and juice with added β -glucan (4 g β -glucan in each) increased satiety more than control biscuits and juice without β -glucan. Given the variance in experimental conditions (different β -glucan dose, various MWs and food sources of the β -glucan, different dosing protocols, and diverse types of subjects), ranking the satiating power of β -glucan is still not possible at this stage. Moreover, another concern to be addressed in future studies is the type of control to use. No dietary fibre that may function as a control for satiety studies has actually been identified. It has recently been recommended that the control should be determined by the research question, i.e., if a specific type of fibre influences the physiological outcome is of interest, then the control should be the same diet or meal without that specific fibre (Pountanen et al. 2018). However, if the research question aims to assess the influence of a particular fibre characteristic and its relation to a mechanism of action, then various forms or levels of physiochemical characteristic of one particular fibre source should be compared with each other, thus the control should then contain the same amount of fibre as the test groups but with different characteristics. A good example of such an approach is seen in the study by Juvonen et al. (2009), where β -glucanase was used to alter the viscosity of two drinks containing 5.1 g oat β -glucan.

It is important to acknowledge that variability in energy intakes may have resulted due to menstrual cycle status (Asarian and Geary 2013), which was not monitored or controlled in female subjects in this study. Although literature is conflicting with regards to the influence of reproductive hormones on energy intake (Davidsen et al. 2007), luteal hyperphagia cannot be ruled out as a possible facilitator of food intake in females. Energy intake has been reported to increase as much as 90 – 500 kcal per day during the pre-menstrual phase compared to energy intake during the follicular phase (Li et al. 1999; Pelkman et al. 2000; Cross et al. 2001). Furthermore, it has also been proposed that insulin responsiveness is modified by sex hormones, for example oestradiol (Bisdee et al. 1989; González et al. 2000) and that insulin sensitivity is lower in the luteal phase than in the follicular phase (Valdes and Elkind-Hirsch 1991; Escalante and Alpizar 1999). Thus, two-thirds of study subjects were female and it should be considered that a possible eating-inhibitory effect of β -glucan may have been masked by increases in food consumption in females who participated during pre-menstrual phases. It should be noted that there is a lack of studies that have investigated whether sex hormones interact with appetite peptides to control changes in appetite over the course of one menstrual cycle (Davidsen et al. 2007), knowledge of which would be instrumental for appetite research.

6.1.2 Influence of viscosity on GLP-1 response

Intestinal satiation refers to the exposure of the intestinal mucosa to nutrients which trigger the secretion of peptide hormones known to control eating via neuroendocrine pathways. It has been speculated that because viscous dietary fibres increase the viscosity of digesta in the small intestine (Tosh et al. 2010), they prolong small intestinal transit time and absorption rate of nutrients which increases contact time with enteroendocrine cells and, thus, peptide release (Regand et al. 2011). In addition, high viscous fibres may disrupt proper mixing of food particles and digestive enzymes, resulting in an increased delivery of unabsorbed nutrients into distal parts of the small intestine where the density of GLP-1 and PYY secreting L-cells is highest (Kristensen and Jensen 2011; Rebello et al. 2016b). Indeed, Beck and colleagues reported

that CCK and PYY increases linearly with increasing amounts of oat β -glucan (Beck et al. 2009a; Beck et al. 2009b). For PYY, there was a significant dose response relationship between grams consumed and PYY AUC ($r^2 = 0.994$, $p = 0.003$). The effect was most pronounced with doses of 4 to 6 g at a late postprandial phase at 4 hours after intake, as a function of both viscosity and concentration (Beck et al. 2009b). However, another study found significant peak increases in PYY as soon as 30 minutes after a 3 g intake of β -glucan from enriched bread (Vitaglione et al. 2009) but there was no effect over a 5 hour postprandial period in 14 healthy subjects who consumed a 10.6 g oat-fibre-enriched bread (Weickert et al. 2006). Juntunen et al. (2002) demonstrated an increase in post-prandial GLP-1 at 120 and 150 minutes following 5.4 g β -glucan containing rye bread. In contrast, a study by Ames et al. (2015a) reported no effect of barley fibre enriched tortillas on postprandial GLP-1 secretion, doses of which ranged from 4.5 g to 11.6 g β -glucan. Moreover, when the natural viscosity of a 300 mL beverage containing 30 g oat bran concentrate (including 5.1 g soluble fibre) was reduced by β -glucanase treatment, 180 min AUC profiles for CCK, PYY and GLP-1 were increased rather than decreased when compared with the high viscous isocaloric control drink (Juvonen et al. 2009). The high-viscosity beverage was still rated as more filling than control, and although there was no difference in *ad libitum* eating this finding suggests that increased viscosity does not favour CCK, PYY and GLP-1 secretion and that oat bran affects appetite independent of GI peptide secretion, which is in line with current study findings. A small but significant decrease in plasma GLP-1 at 90 minutes with oat β -glucan suggests that firstly, high viscous oat β -glucan does not favour activation of enteroendocrine cells and thus GLP-1 secretion, and secondly, oat β -glucan beneficially modulates appetite independent of increased plasma GLP-1, at least under the current acute study conditions. Assuming that oat β -glucan slowed GE 0-90 minutes after loading, an explanation for the decrease in plasma GLP-1 is provided by studies showing that the secretion of GLP-1 and other GI peptides is dependent on intestinal caloric load, with higher loads resulting in larger responses (Pilichiewicz et al. 2007). However, an effect on later phase GLP-1 secretion due to increased delivery of unabsorbed nutrients into distal intestinal parts should not be dismissed, as the current study did not measure GLP-1 for more than 90 minutes, which is a limitation of the study. Therefore, sampling periods should consider the late-phase release of GLP-1 and

sample over a period of ≥ 240 minutes (Elderick et al. 2017). Future studies should also take into consideration the potential influence of mechanistic target of rapamycin (mTOR) activation in intestinal L-cells following meal intake. Xu et al. (2015) suggest that mTOR activation in L-cells leads to increased GLP-1 secretion in response to meals, thus, this may also explain why the viscous gut contents in the current study did not favour GLP-1 release from enteroendocrine cells.

6.1.3 Test meals and Visual Analogue Scales

With regards to sensory aspects of food, a thick and creamy mouthfeel is reported to induce greater and more prolonged reduction in hunger compared to thinner products (Bertenshaw et al. 2013). A possible drawback to the current study may be that a Greek-style yoghurt was used, thus from an oro-sensory perspective, may have masked the satiating effect of the oat bran added to the intervention test breakfast. Using a thinner yoghurt may have been a more ideal vehicle for β -glucan ingestion in the current study. Nevertheless, β -glucan used in this study was a concentrated extract of oats, which allowed enrichment of the test breakfast without significantly altering macronutrient composition compared to the control. As a result the macronutrient contents of both test breakfasts were very well matched, and differed only by their fibre content, which is key element in appetite study design. In addition, the study included an adequate sample size ($n=33$) and a crossover design which helped reduce subject variation in the appetite markers measured. Other acute appetite studies that have evaluated the efficacy of β -glucan to alter appetite sensations have tested as few as 12 subjects (Hlebowicz et al. 2008; Beck et al. 2009a; Lyly et al. 2009; Hartvigsen et al. 2014), with sample sizes determined by VAS outcomes from previous literature.

The sensory experience of eating is an important determinant of food intake control, often attributed to the positive hedonic response associated with certain sensory cues (McCrickerd and Forde 2015). As well as considering post-ingestive influences on satiety, it is important to consider aspects of satiety that could be attributed to the consumer's experience of consuming the food before it is processed by the GI system. The

taste, smell and texture of a food all contribute to the representation of its flavour, but food texture has been isolated as a sensory component of food that plays a key role in satiety (Chambers et al. 2015). This is because oro-sensory signalling is refined by a lifetime of food experiences, when it is learnt that certain properties of a food's flavour are better predictors of the presence of nutrients than others (Le Magnen 1955). Viscous foods are invariably nutrient rich and consumed in the context of hunger, unlike fluids that may or may not contain nutrients and are primarily consumed for their thirst-quenching properties. Food texture, therefore, may serve as a reliable predictive cue for future sensations of satiety (Davidson and Swithers 2005), shaping expectations about the affect a food will have on appetite. Textured foods require chewing which will slow rates of consumption and enhance oro-sensory exposure time (Zijlstra et al. 2008). The mechanical processing of food in the mouth might be one way in which the nutrient content of a food is estimated. Indeed, mastication has been associated with satiety-related cognitions (Forde et al. 2013), preparatory cephalic phase responses (Li et al. 2011) and appetite peptide release (Katsuragi et al. 1994; Li et al. 2011), but relationships with satiety signals are not always reported (Teff 2010; Mattes and Considine 2013).

The most compelling evidence for a pre-ingestive explanation for the effect of texture on satiety was by Cassady et al. (2012). In their well-designed study, participants consumed a preload on four occasions: on one day they consumed a cherry liquid beverage and with a convincing cover-story they were led to (falsely) believe that this would turn to a gel in their stomach ("liquid-solid"); on another day they consumed the same beverage and were (correctly) told that this would remain a liquid in their stomach ("liquid-liquid"); in the third condition cherry-flavoured gelatine cubes were consumed which they (correctly) believed would turn to liquid in their stomach ("solid-liquid") and in the final condition they consumed the same gelatine cubes and were (incorrectly) informed that they would remain in this form in their stomach ("solid-solid"). Oral exposure time was held constant on all days, and the researchers chose gelatine jelly as the oral solid as this would rapidly liquidise in the stomach once consumed. To ensure that the actual gastric load was exactly matched in all conditions participants consumed capsules of gelatine on sensory "liquid" days and water and maltodextrin on sensory "solid" days. When the sensory experience of the preload was solid

(“solid-liquid” and “solid-solid” conditions) ratings of hunger were lower, GE was slower, insulin and GLP-1 release increased, ghrelin decreased, and subsequent *ad libitum* food intake was lower compared to the days when the sensory experience was liquid. In addition, the false belief that the preload would turn to a solid in the stomach further augmented these satiety responses. These findings provide strong support for satiety enhancing effects of pre-ingestive sensory and cognitive information. With regards to the current study, subjects were responsible for adding milk to the Rice Krispies cereal to avoid an unpleasant consistency of the cereal. Nevertheless, palatability ratings were significantly less satisfactory following the β -glucan test breakfast, in addition to taste and visual appeal. Therefore, memory or other cognitive effects may have influenced subsequent eating as suggested by some studies (Johnson and Vickers 1992; Yeomans et al. 2001). However, palatability is just one aspect of the sensory experience. Sensory cues based on a food's sight, smell, taste and texture are operational before, during and after an eating event (McCrickerd and Forde 2015). The initial experience of eating is important because humans learn to eat in response to sensory cues, by forming associations between the early experience of a food's sensory characteristic and the post-ingestive effects of nutrient delivery. Sensory characteristics that consistently cue nutrient delivery acquire meaning and can be used to predict the consequences of consumption (Sclafani 1997). This learned integration of pre-ingestive and post-ingestive signals can be expressed as increased liking for nutrient-rich foods and explicit beliefs about a food's potential satiating power (Brunstrom 2007), which in turn modify food selection and intake. Cephalic phase responses form part of the rapid conditioned physiological response to food-related stimuli, such as salivation (Wooley and Wooley 1973), gastric acid secretion (Feldman and Richardson 1986) and the release of some GI hormones (Smeets et al. 2010). These responses are triggered upon the sight, smell and taste of a food, to optimize nutrient processing throughout the GI tract (Feldman and Richardson 1986). This indicates that sensory signals present before and during consumption play a functional role in optimizing energy intake regulation, beyond palatability, by informing the perceptual and physiological response to nutrient selection and ingestion.

Fortunately, there were no differences in ratings of aftertaste and smell between breakfasts consumed in the present study, which are both characteristics that could have influenced eating behaviour at the subsequent

ad libitum test meal. From verbal feedback (data not presented), several subjects found the combination of oat bran powder and Greek-style yoghurt a lot less palatable than the Rice Krispies cereal. Perhaps this was because the colour of the Greek-style yoghurt was not white (as expected), but instead was a cream-yellow colour. As this did not meet their expectations, subjects may have suspected the Greek-style yoghurt to have been manipulated, particularly if their first visit to the study was the control breakfast. A drawback of the study was that the sensory feedback collated from study breakfasts was not for each individual breakfast component, instead ratings were recorded for the complete breakfast and distinctions could not be made between the cereal and yoghurt.

The *ad libitum* lunch offered to subjects was acceptable across all sensory characteristics measured, therefore it can be assumed that the ham sandwiches eaten were pleasurable to study subjects, i.e., subjects consumed ham sandwiches because as a food item they were liked. A drawback to using such single-item *ad libitum* lunch is the possibility of early termination of eating not because subjects felt ‘comfortably full’ but because of SSS. Visual heuristics such as ‘plate cleaning’ and portion cues can direct food intake and override physiological feedback during consumption and thus should also be considered. For example, the tendency to clear one's plate when eating was associated with increased food intake during a lunchtime meal in a recent study by Sheen et al. (2018), which is in agreement with several other studies that reports people tend to consume more when a food is served in larger compared with smaller portions (Rolls et al. 2002; Wansink et al. 2005; Wansink and Kim 2005; Rolls et al. 2010). Additionally, people do not always passively overconsume in response to visual portion cues (Rolls et al. 2007). It has been shown that the effect of expected satiety can persist well into the inter-meal interval (Brunstrom et al. 2011). Some elements of food selection are far more active and based on previous experience. Green and Blundell (1996) demonstrated that aspects of a food's appearance are used to evaluate how filling a food will be before it is consumed. It is likely that all subjects in the current study had consumed ham sandwiches before, therefore it is possible that they had a preconceived idea of how many ham sandwich quarters would equate to consuming an amount that they would typically consume, i.e., four quarters being equal to one sandwich.

This may have influenced their eating behaviour, however as mentioned the current study followed a cross-over design, thus subjects consumed the *ad libitum* lunch on both visits under the same feeding conditions. As previously discussed (**Chapter 3, section 3.1.1**), satiety is most commonly measured subjectively by using VAS, which have been reported to have good repeat reliability between groups, allowing similar and compatible results to be produced in other clinical trials. Yet despite their validity, there are limitations to this method. Inter-individual differences which can appear in the use and understanding of VAS (Raben et al. 1995), along with and the high variability in experimental designs (inter-meal interval, type and energy of preloads, etc.) have to be considered (Ortinou et al. 2014; Pentikäinen et al. 2014; Leidy et al. 2015). Murray and Vickers (2009) highlighted the complexity of hunger and fullness sensations of consumers qualitatively using focus groups. They concluded that sensations of mental hunger and physical fullness overlapped, which provided evidence that the overall constructs of hunger and fullness may not be simple, and possibly opposing. Reducing inter-individual variability of VAS could improve the precision of satiety assessment. According to EFSA (2012), in the context of satiety claims, it is possible to make claims on changes in appetite ratings, however, '*the beneficial physiological effect of changing appetite ratings depends on the context of the claim*'. Therefore, evidence on changes in appetite ratings alone may not be sufficient for the scientific demonstration of the claim (EFSA 2012; Halford and Harrold 2012). It could therefore be interesting to establish a methodology not intended to replace traditional VAS but to include measurement of food intake while making quicker and efficient screening of several products according to their satiety power possible. Selecting sensory panelists who have experience of evaluating feelings about foods, and then training these panelists to apply their experiences to assessing appetite feelings may provide more discriminative results with regards to the outcomes of subjective appetite ratings. Lesdéma and colleagues (2016) trained a group of subjects to evaluate appetite sensations using VAS, with focus on understanding the vocabulary to define appetite, manipulating scales and using personalised scales to evaluate a range of CHO-rich snacks. During training, subjects personalized their own satiety lexicon that was then used to assess CHO-rich foods. In trained subjects, Lesdéma et al. (2016) report that using a personalized scale to rate CHO-rich foods

showed a stronger discrimination of satiety power when compared to untrained subjects, with good reproducibility between methodologies. Although the method was effective in identifying a product with low satiety vs. high satiety effectively in the trained subjects, the ranking of products in order of their satiety was not the same when trained and untrained subjects were compared. Additionally, using trained subjects would limit the external validity of the findings. Nevertheless, using satiety lexicon made up of common vocabulary and illustrated by personal examples requires further validation, which may aid to obtain an efficient commonly built tool for assessment of subjective appetite.

6.2 Glycaemia and insulinaemia

As expected, consumption of β -glucan at breakfast significantly blunted the post-prandial blood glucose and insulin responses which are in line with a large number of previous studies (Wolever et al. 2010; Tosh et al. 2013). This is most likely due to a delay in GE and subsequent glucose absorption, although, a weakness of the current study was that this was not directly assessed. However, it was shown that the test meal rich in oat β -glucan showed substantially higher viscosity than the control meal, which supports this hypothesis.

It has long been established that post-prandial glucose response to CHO-rich meals is not determined by the amount of available CHO alone, instead the proportions of macronutrients, particularly protein and fat, as well as food microstructure, can dictate the rate of glucose absorption (Tosh et al. 2013). A range of soluble fibres, including β -glucan, have been shown to improve glycaemic responses, with each gram of β -glucan reported to decrease glycaemic index by four units (Mäkeläinen et al. 2007).

What is less well defined is the best suited food vehicle in which to deliver β -glucan to the diet as the effects of food processing are frequently questioned with regards to maintaining the efficacy of soluble fibre in oat and barley products. A wide variety of food formats are used in clinical studies. According to systematic review findings by Tosh et al. (2013), all studies included that used > 4 g β -glucan from isolates or extracts

significantly reduced the AUC in glycaemic responses, with an average reduction of 58 ± 39 mmol/min/l (Wood et al. 1990; Braaten et al. 1991; Hallfrisch et al. 2003; Mäkeläinen et al. 2007; Panahi et al. 2007). The efficacy of β -glucan supplements is most likely due to it being easier to achieve higher concentrations of β -glucan in a meal. The dose for these studies ranged from 4 – 11.3 g β -glucan per meal. Aman et al. (2004) and Regand et al. (2009) report that depolymerisation of β -glucan occurs during the processing of bread and pasta, therefore it is suggested that processing techniques have the ability to influence the functionality of β -glucan with regards to attenuating glucose responses. However, there is evidence of breads containing at least 4 g β -glucan to have shown significant decreases in glycaemic responses (Liljeberg and Bjork 1994; Juntunen et al. 2002; Östman et al. 2006; Thondre and Henry 2009; Finocchiaro et al. 2012). Equally, in pasta products, studies have shown favourable outcomes for post-prandial glycaemia (Holm et al. 1992; Chillo et al. 2011; Aldughpassi et al. 2012). In studies that have incorporated at least 4 g β -glucan into muffins, there have been significant reductions in blood glucose. Muffin batter has a high water content allowing β -glucan to solubilize, and thus disperse through the crumb (Tosh 2007; Lan-Pidhainy et al. 2007). Reduction in glucose responses have also been reported following consumption of at least 4 g β -glucan in granola, muesli and breakfast cereals (Tosh et al. 2013). It is possible that because moisture content of these meals are kept low during processing, this may favour β -glucan as hydration is inhibited. Therefore, the main concern with regards to the effects of processing on efficacy potentially lies in maintaining the MW of the β -glucan.

The current study used a commercially available oat bran powder (OatWell™), which was incorporated into cereal and yoghurt, both of which were consumed without heat treatment. Despite significant reductions in post-prandial glucose at 30 minutes (6.0 mmol/L vs. 6.5 mmol/L), it was anticipated that a larger difference in glucose responses following the β -glucan breakfast may have been seen, particularly as 4 g of β -glucan was consumed. A possible explanation for this may be due to the CHO content of the test breakfasts (39 g) being slightly higher than the 30 g available CHO recommended by EFSA (2011). However, despite no changes in glucose AUC between breakfasts in the current study, there was a flatter peak rise and thus a sustained glucose uptake following the breakfast containing 4 g β -glucan. The 4 g of

β -glucan was effective in attenuating fluctuations in both insulin and blood glucose responses, which is in line with a large body of evidence supporting EFSA health claim of 4 g of β -glucans from oats or barley for each 30 g of available carbohydrate should be consumed per meal.

Oatmeal elicits a lower glycaemic response than most other types of ready-to-eat and cooked breakfast cereals when comparing equivalent amounts of available CHO (Tosh and Chu 2015; Wolever et al. 2015). Since most (Wood et al. 1994; Björklund et al. 2005; Maki et al. 2007; Panahi et al. 2007) but not all (Juntunen et al. 2002; Beck et al. 2009a; Juvonen et al. 2011) studies have demonstrated that the addition of high MW oat β -glucan to test-meals reduces glycaemic responses in human subjects, Wolever and colleagues (2018) recently investigated the impact of adding a small amount of oat β -glucan to instant oatmeal in order to identify if this would further enhance its glycaemic effect. Wolever et al. (2018) aimed to identify the minimum amount of oat bran β -glucan required to be added to a standard 27 g serving of instant oatmeal (with a natural oat β -glucan content of 1.2 g) that would elicit a 20% reduction in glucose AUC when compared to a β -glucan-free control meal (cream of rice). A 20% reduction in glucose AUC has been suggested by Health Canada (2013) to be the minimum reduction required to support a claim related to a reduced glycaemic response. Following a randomized, controlled fashion, glycaemic responses of 40 subjects who consumed instant oatmeal, instant oatmeal with 0.2, 0.4, 0.8 or 1.6 g oat β -glucan from oat-bran, and an available-CHO matched portion of cream of rice were compared. Results showed a correlation between grams of oat β -glucan consumed with mean glucose AUC and mean glucose peak-rise. Regression analysis suggests that for each gram of oat β -glucan consumed, AUC and peak-rise reduced by 7 and 15%, respectively. The authors report that in order to achieve $\geq 20\%$ reduction in AUC compared to the cream of rice, 1.6 g oat β -glucan had to be added to the oatmeal, but to achieve a 20% reduction in peak-rise only 0.4 g oat β -glucan was required. Not only does the study by Wolever et al. (2018) show that a total of 2.8 g oat β -glucan (1.2 g from oatmeal and 1.6 g β -glucan from oat bran) is effective in attenuating glucose responses, it is novel in nature as it highlights the practical implications of meeting the EFSA claim for reducing glycaemic responses. In the current study, 14.7 g of OatWell™ oat bran powder was consumed to achieve 4 g β -glucan, whereas Wolever and colleagues used 0.72, 1.43, 2.86 and 5.72 g oat bran powder

to achieve β -glucan doses of 1.4, 1.5, 2, 2.8 g within the oatmeal food matrix, respectively. Firstly, it can be suggested that using a smaller quantity of oat bran powder may make meals more palatable, and thus from a consumer perspective easier to consume, which was a drawback of the current study. Secondly, oatmeal contains an overall beneficial nutritional profile, i.e., delivering protein, magnesium, thiamin and mono- and polyunsaturated fats to the diet as well as oat β -glucan. From a health perspective, adopting the approach of Wolever and colleagues would be nutritionally more beneficial for improving the quality of diets. Although satiety was not the focus of the study by Wolever et al. (2018), protein from oatmeal may work synergistically with β -glucan to increase satiety, which is an avenue for future research.

The health claim by EFSA (2011) for attenuating glucose responses, “*4 g of oat β -glucan per 30 g of available carbohydrates*”, can also be taken from the perspective of 0.133 g oat β -glucan is required per gram of available CHO to obtain a reliable effect on glycaemic response. Therefore, amounts of oat β -glucan <0.133 g per gram available CHO would be considered low. From the literature identified, that investigated >0.133 g β -glucan per gram available CHO, glycaemic responses were reduced in all studies (Wood et al. 1990; Wood et al. 1994; Regand et al. 2011). Conversely, in studies identified that used < 0.133 g there was no effect on glycaemic responses (Biörklund et al. 2005; Mäkeläinen et al. 2007; Tosh et al. 2008). In the current study, there were 4 g oat β -glucan to 39 g available CHO, thus 0.102 g oat β -glucan per gram of available CHO. Although only fractionally lower, considering the evidence that reports <0.133 g has no effect on glycaemic responses, the current study is in line with the findings of Wolever et al. (2018), demonstrating that as little as 0.102 g oat β -glucan per gram available CHO can reduce post-prandial glucose responses.

The observation of reduced insulin release was a positive outcome, most likely through the delayed rate of glucose delivery. Insulin resistance is an integral feature of the MetS and is a major predictor of the development of T2DM (Jung and Choi 2014). It has long been recognized that obesity is associated with T2DM, and the major basis for this link is the ability of obesity to induce insulin resistance. Karpe and Tan (2005) suggest that adipose tissue is exquisitely insulin sensitive and hyperinsulinaemia may therefore lead

to a constant lipolytic inhibition in adipose tissue. Therefore, any ingredient which decreases insulin secretion may be helpful for attenuating obesity promoting effects. Except for cheese, dairy products, including whole and skimmed milk, yoghurt, ice-cream and fermented milk products, have been shown to have insulinotropic properties (Östman et al. 2001; Hoyt et al. 2005; Hoppe et al. 2009). Certain amino acids (tryptophan, leucine, isoleucine and glutamine) are insulinogenic, hence, it has been hypothesized that elevated concentrations of these amino acids in milk and milk products may underlie its insulin-stimulating capabilities (Schmid et al. 1989; Newsholme et al. 2007; Zhao et al. 2016). The hyperinsulinemia resulting from milk and dairy consumption may be considered a beneficial and even protective effect for regulating blood glucose levels, particularly in individuals with elevated levels or those with T2DM (Nuttall and Gannon 1988). Despite this, consumption of milk and dairy products and the resultant hyperinsulinemia may produce less-than-desirable long-term effects in healthy individuals, including insulin resistance (Tucker et al. 2015). Research in animals and humans suggests that regular hyperinsulinemia can lead to specific defects in the non-oxidative glycogen synthesis pathway, which may lead to the development of insulin resistance (del Prato et al. 1994; Juan et al. 1994). In the current study, consumption of 4 g oat β -glucan in a milk and yoghurt matrix significantly reduced post-prandial insulin release in healthy individuals, which could potentially help reduce hyperinsulinemia in response to protein and CHO-containing food consumption in this population.

6.3 Adverse reporting and practicality of intervention

Increased flatulence and bloating, in addition to changes in stools, were reported by subjects following both control and intervention breakfasts. However, the most frequent adverse effects were reported following the breakfast containing β -glucan. Since oat β -glucan has a strong water-binding and viscosity-thickening capacity, it is likely that the adverse effects reported following the β -glucan breakfast can be attributed to the bulking action of β -glucan in the digesta, driven by the water-absorbing capacity of β -glucan. Although GE was not measured in the current study, it is feasible that since food transit through the small intestine

depends on GE rate (Müller et al. 2018) changes in GI habits were reported because of the slower transit of undigested food through the small bowel and subsequent fermentation by colonic bacteria to produce gas as a by-product. Interestingly, unwanted GI effects were reported following breakfasts without β -glucan, therefore it is important to acknowledge that β -glucan was not responsible for facilitating GI changes following the control breakfast. The white bread consumed at the *ad libitum* lunch contained 1 g of fibre per slice (40 g), therefore, it may be possible that GI changes may have occurred as a result of white bread consumption.

Health benefits aside, changes in GI habits are often perceived negatively with consumers, and may prevent the consumer from purchasing such a product. As this was an acute study, it is not possible to determine whether the changes reported in GI effects were a one-off. Perhaps subjects who experienced unwanted GI changes would avoid consuming this particular fibre supplement, despite its health benefits. Surprisingly, according to literature, no adverse GI effects have been reported following testmeals with ≥ 5 g β -glucan (Beck et al. 2009a; Juvonen et al. 2011; Clegg and Thondre 2014).

The condition of use for the glucose lowering effect of β -glucan is likely to be difficult for food processors to meet. From a practical point of view, consuming 4 g of β -glucan from natural sources, for example from porridge oats, would require consumption of approximately 3-4 bowls of porridge, and thus would be excessive in CHO content (>70 g CHO without milk) to meet the desired health claim. Not only would it be unrealistic to consume, consuming 4 g oat β -glucan from oats would be at in excess of 500 kcal. Therefore, the convenience of supplementing meals with concentrated oat bran can be an efficient way to deliver adequate β -glucan simultaneous to increasing fibre intake, which is below the recommendation for adults of 30 g per day in UK, at a low kcal expense (40 kcal).

Evidence from Brunstrom and Rogers (2009) suggests that many consumers select quantities of foods on the basis of anticipated satiety benefits, i.e., choosing smaller (volume) portion sizes of foods expected to deliver more fullness per kcal, or, in other words, how satisfying eating that food would be. Moreover, Brunstrom and Rogers (2009) found that individuals gave more value to (but did not like more) foods with

this higher energy-adjusted 'expected satiation'. This perhaps reflects the learned anticipation of the satisfaction that a portion of food will deliver, based on habitual experience with such foods. Thus, given the chance, individuals will choose foods that provide them with just the right level of satisfaction for the context. What is not established is how the consumer might adapt in the long term, or if the benefits of satiety enhancement would be sustained over time, which may indeed highlight the potential benefits of enhanced satiety described by Hetherington et al. (2013). Since findings suggest that consumers may opt for more satiating choices, despite whether or not they like eating the particular food choice, it should be considered that the oat bran consumed in the current study could be incorporated into the diets of those who seek health benefits (cholesterol-lowering, glucose attenuation, fullness) even though they might not enjoy the product.

The clinical implications of reducing postprandial glucose oscillations is beneficial for the population as a whole. Flattening the glucose response by reducing peak-rise reduces post-prandial glucose fluctuations is useful for individuals who have pre- and/or are diabetic. In patients with T2DM, a high magnitude of glucose fluctuations was associated with increased oxidative stress independently of overall glycaemic control as assessed by HbA1c (Monnier et al. 2003; Monnier et al. 2006). For individuals without impaired glucose control, flatter glycaemic responses have been associated with reduced postprandial NFκB activation in white blood cells, suggesting enhanced anti-inflammatory effects (Dickinson et al. 2008).

The current study was performed in a controlled laboratory setting where a designated testing room was reserved for the consumption of the test breakfasts and *ad libitum* lunch. This experimental setting, commonly used in postprandial studies, guarantees quantitatively accurate and reliable measurements of appetite sensations and energy intake. It also ensures the sensitivity to the experimental manipulations without interference of multiple environmental factors. Previous studies have demonstrated that a variety of factors in the environment may distract or interfere with the eating occasion (Wansink 2004; Wansink 2005). Particularly, the company of other people clearly affects the eating behaviour of subjects (Salvy et

al. 2007; Hermans et al. 2012; Cruwys et al. 2015). Although laboratory settings, in which several confounding factors can be controlled for, may be a proper procedure in many ways, it raises the question whether the results can be extrapolated into real life situations. Simultaneously, in appetite research the optimal experimental protocol likely remains elusive due to the complex nature of eating behaviour (Blundell et al. 2010). As the purpose of this study was to investigate the effects of experimental test products on energy intake, appetite and postprandial physiology, a controlled laboratory condition free of ‘distracting’ variables was an appropriate choice. If considered worthwhile for further investigation, more realistic settings can be applied in future. Perhaps conducting a feasibility study, where subjects can consume oat β -glucan at breakfast in their own home environment and record subjective ratings of appetite could be explored, however identifying changes in food intake could only be measured by food recording, as blinded *ad libitum* eating would be more difficult outside the laboratory setting. Whether the effects seen on subjective fullness and satiety, along with glucose, insulin and GLP-1, are also seen over time warrants investigation.

Along with five portions of fruit and vegetables and consumption of wholemeal bread, SACN (2015) advise consuming a portion of high fibre breakfast cereal to help achieve the recommended daily intake of 30 g fibre per day. Yet according to the most recent findings from the Scottish Health Survey (2017), only 28% of adults consume high fibre cereal at least 5-6 times a week. Whole food sources of nutrients are generally considered preferable to fortification or supplementation with isolated food components, as whole grains may supply additional beneficial nutrients beyond that of fibre alone, such as B vitamins and polyphenols. However, fibre supplementation can be convenient, and in conjunction with a balanced diet, concentrated forms of fibre, such as oat bran powder, could help individuals meet their DRV for fibre intake. Adding small amounts of oat bran powder to meals throughout the day could increase daily fibre consumption for the general population.

Chapter 7

7.0 Effects of a six-week intervention with novel β -glucan-enriched oatcake snacks on daily energy intakes, body composition and markers of metabolic health in overweight and obese individuals: a pilot study (Study B): Methodological Considerations

As the worldwide obesity epidemic continues to escalate, the food industry tries to provide options for consumers who embrace diets composed of ‘healthy foods’, particularly those which will play a role in weight loss and weight management. Firstly, research identifies dietary patterns that provide epidemiological evidence for health benefits, for instance, linking the Mediterranean diet with lower incidences of CVD (Bonaccio et al. 2012; Tektonidis et al. 2015). Secondly, the specific diet is broken down and its individual components are investigated, such as olive oil or vegetable content. These foods are then dissected further into basic components, i.e., omega 3 polyunsaturated fats or antioxidants. Incorporating these ingredients into a diet may or may not provide the same health benefits as the original diet. Yet including a beneficial ingredient into a different cultural food system may give at least a few health benefits to consumers and opportunities to food industry to capitalise on.

Epidemiological studies show an inverse relationship between fibre intake and body weight (Slavin 2005; Tucker et al. 2009; Lattimer and Haub 2010). As part of food, fibres are present in soluble (fermentable) or insoluble (non-fermentable) form, both of which have been unable to show definitive effects on eating. In order to identify the advantages of epidemiological studies, more focus is needed to understand the physical features of fibre, the food delivery system, and how they may work synergistically to impact mechanisms associated with body weight, such as food intakes, over longer periods of time. In short term studies, it has been suggested that a minimum of 4 g β -glucan is required to influence the release appetite hormones (Granfeldt et al. 2008; Beck et al. 2009a). Yet the very nature of acute studies will only define short term effects (under very controlled conditions) and therefore studies of a more than 24 hours are required.

As well as exposure time, the way in which β -glucan is delivered to the diet also requires consideration, as the bioactivity of β -glucan may be reduced in some foods. Andersson et al. (2004) report that during the breadmaking process β -glucan was degraded by endogenous β -glucanases in barley and wheat flour. They highlight that mixing and fermentation time can inhibit the functionality of β -glucan, as lowering the viscosity of β -glucan may reduce its clinical effectiveness. On the other hand, 10 g high dose β -glucan consumption showed that a lower viscosity drink (which had been treated with β -glucanase to reduce viscosity) increased levels of CCK and GLP-1 compared to the (untreated) high viscosity version of the drink (Juvonen et al. 2009). However, it is important to bear in mind that such studies do not predict what may happen when β -glucan fibre is consumed over time – acute changes in appetite related hormones do not necessarily translate into appetite and weight changes over a longer period of time.

7.1 Body composition

Body mass index is widely used for routine characterization of weight status in epidemiology, clinical nutrition and research due to its simplicity and ease to perform. In adults, BMI is a stature-independent measure of body weight and a surrogate measure of total body fat. BMI is used to classify subjects into the categories ‘underweight’ ($< 18.5 \text{ kg/m}^2$), ‘normal weight’ (> 18.5 and $< 25.0 \text{ kg/m}^2$), ‘overweight’ ($> 25 \text{ kg/m}^2$) or ‘obese’ ($> 30 \text{ kg/m}^2$) (WHO 2000).

Scientifically, BMI is a crude means to characterize disease risks. Not only that, meta-analyses suggest that a high BMI-associated disease risk does not necessarily result in higher mortality (Flegal et al. 2013; Winter et al. 2014). Contrary to class II and III obesity, overweight and class I obesity did not increase the mortality risk (Flegal et al. 2013), which ultimately questions the true value of BMI as a health risk surrogate. Strictly speaking, obesity is not defined as an excess of body weight relative to height but as a state of increased adiposity of enough magnitude to produce adverse health consequences, and it is for this reason that BMI has been scrutinized over the last few years.

Body weight and BMI are most commonly used to monitor the effects of different strategies for weight management. The process of weight loss shows variability from individual to individual; weight loss is often slow and gradual, and this phenomenon cannot be explained by body weight or BMI alone. With regards to energy balance, although now considered an oversimplification, a common rule states that reducing energy intake by 500 kcal per day (or 3,500 kcal per week) will lead to a weight loss of 0.45 kg per week (Wishnofsky 1958; Hall 2008). However, in case of a negative energy balance and perfect adherence of subjects to diets, there is considerable variance between individuals in weight reduction, and furthermore weight loss is not linear over time (Müller et al. 2016). In relation to weight loss-associated changes in body composition, changes in energy content in relation to changes in body weight differ between subjects as well as during the time course of weight loss (Müller et al. 2015). Losses in either fat free mass or fat mass associated with a negative energy balance (and therefore the slight loss of fat free mass with dieting strategies) provide a mechanistic approach to explain inter-individual variances in weight loss, thus highlighting the importance of their monitoring in weight management.

7.1.1 Direct measurements

Various indirect two compartment methods for measuring body fat in a clinical research setting are available, including anthropometry (skinfold thickness), densitometry, radiography (dual energy X-ray absorptiometry; DEXA, and computerised tomography; CT) in addition to bioelectrical impedance analysis (BIA). For some time, underwater weighing was considered the ‘gold standard’ two-compartment method for determining fat versus fat-free mass (Duren et al. 2008). Measurements of mass and volume are acquired and used to calculate density before equations are used to determine body fat percentage (BF%; Siri 1993). There are however several limitations to this method, which requires individuals to fully exhale whilst submerged underwater in order to establish residual lung volume. A more acceptable method, which is based on the same principles, is air displacement plethysmography (ADP) which uses a sealed chamber to measure volume displaced by air rather than water. The two methods have been shown to compare well across a wide population, suggesting that ADP could be an alternative and equally reliable method (Biaggi

et al. 1999; Demerath et al. 2002). Yet with technological advances and medical imaging, DEXA has gained popularity as an alternative method for measuring body composition. A review of the accuracy of DEXA in the measurement of body composition concluded that despite technological advances, DEXA appears to be less accurate at extremes of fatness when compared with the four-compartment (4C) method (Toombs et al. 2012). Despite this, DEXA has the added advantage of allowing analysis of BF% in different body segments, providing more detailed assessment of risk with information on distribution and location of adiposity. Cost of equipment and exposure of individuals to radiation (albeit very small dose) and restriction of DEXA use to certain populations, are all main drawbacks to using DEXA to determine body composition. Both DEXA and ADP completely lack portability, require a stable temperature-controlled environment and are considerably expensive (von Hurst et al. 2016).

Bioelectrical impedance analysis monitors are very portable and easy to use, which makes them ideal for field studies, ultimately offering a more discriminatory measure than BMI. Bioelectrical impedance analysis measures body composition by running a series of alternating electrical currents with a frequency ranging from 5 kHz to 1 MHz through the body. These signals interact differently with body cells and fluids, transmitting the potential difference (voltage) back to the BIA device. The resulting data is given as impedance (ohms) which is a combination of resistance and capacitance data. Bioelectrical impedance analysis measurements are determined by the resistance of the body to electrical current flow between points of contact on the body and correlates well with total body water measurements (Erceg et al. 2010). Bioelectrical impedance analysis exhibits greater resistance to electrical current flow in fat tissues than fat-free tissues because of their differences in water content. In terms of fat-free mass, BIA has also been shown to be a good predictor, when compared to DEXA ($r=0.85-0.88$; Roubenhoff 1996). Bioelectrical impedance analysis provides a reliable estimate of total body water under most conditions. It can be a useful technique for body composition analysis in healthy individuals and in those with a number of chronic conditions such as mild-to-moderate obesity (Powell et al. 2001; Mahon et al. 2007), diabetes mellitus, and other medical conditions in which major disturbances of water distribution are not prominent. Outputs from BIA are

affected by numerous variables including body position, hydration status, consumption of food and beverages, ambient air, skin temperature and recent physical activity, thus a standardised protocol should be followed before BIA is performed (Siddiqui et al. 2016).

7.1.2 Indirect measurements

Using anthropometric measures of visceral obesity is essential to research and clinical practice because it predicts CV and metabolic risks. In addition to BMI, a simple indirect measure of fat distribution is WC. In a European population, central obesity is defined as WC measurements ≥ 94 cm for men and ≥ 80 cm for women (NICE 2017). It is reported that WC is regarded as a stronger marker of health risk than is BMI (Zhu et al. 2002) and it serves as one of the criteria for the diagnosis of MetS (BMJ 2018). Abdominal fat is thought to increase the risk of metabolic disease through a number of secretory factors, including non-esterified fatty acids and adipocytokines including TNF- α and reduced adiponectin. Reduction in WC is associated with an improvement in the circulating levels of these adipose tissue secreted factors (Freemantle et al. 2008). Despite WC being an inexpensive and convenient measurement to perform, this surrogate marker of obesity is beset with problems related to location in obese individuals. There is a non-linear relationship between girth and waist area across a range of adiposity and the lack of geometric similarity suggests that abdominal diameter should be measured without assuming it to be circular. Furthermore, different protocols for measuring WC unsurprisingly show different results, according to Wang et al. (2003) who compared four different protocols. Ultimately, accurate measurements of WC require trained individuals with meticulous precision. Not only that, and most importantly, WC does not distinguish visceral from subcutaneous abdominal adipose tissue (Kuk et al 2005). Pou et al. (2009) reported that WC may misclassify individuals in terms of visceral adipose tissue (VAT), highlighting that other anthropometric measures to correlate with VAT are needed.

Sagittal abdominal diameter (SAD), which measures the anteroposterior diameter of the abdomen, reflects VAT based on the fact that subcutaneous fat is displaced inferiorly by gravity (Kvist et al. 1988). In order to evaluate the usefulness of SAD in predicting visceral obesity, Yim et al. (2010) compared SAD with

other anthropometric measures, such as WC, CT cross-sectional images and transverse abdominal diameter (largest spanned width of abdomen) in a cohort of 5,257 healthy, normal weight men and women. Their study reported that SAD showed a stronger correlation to VAT than WC, BMI, and transverse abdominal diameter in both males and females ($r = 0.804, 0.724$, respectively). Waist circumference showed generally stronger associations to subcutaneous adipose tissue (SAT) than to VAT (men: $r = 0.789$ vs. 0.705 , women: $r = 0.820$ vs. 0.636). Findings concluded that SAD showed the strongest correlation to VAT irrespective of age, sex, and the degree of obesity compared with other anthropometric measures, whereas WC may have a stronger correlation to SAT than to VAT. Despite the fact that this study focussed on normal weight individuals, it highlights the usefulness of SAD in predicting VAT.

Given its suitability for overweight and mild-moderate obese subjects, BIA was chosen to assess body composition in the current study. When planning field studies, practicality is of key importance. Bioelectrical impedance analysis was the most appropriate tool for measurement of body fat percentage and water content in the current study, which was heavily restricted by availability of resources and funding. Portability of equipment and the general low cost of running the study was desirable, hence indirect markers of body composition, such as WC and SAD were utilized.

Chapter 8

8.0 Effects of a six-week intervention with novel β -glucan-enriched oatcake snacks on daily energy intakes, body composition and markers of metabolic health in overweight and obese individuals: a pilot study (Study B): Research Methodology

8.0.1 Study Pre-requisites

Before commencing the study the researcher obtained ethical approval and first aid certification.

Ethical approval for this research study was granted by Queen Margaret University Research Ethics Panel on 22nd September 2015.

8.1 Study design

This experimental study was a double-blind, randomized, placebo-controlled parallel study.

Despite cross-over designs being considered as the ‘gold-standard’ for experimental studies, a parallel group study is a simple and commonly used clinical design which effectively compares two treatments, requiring less commitment from participants. A placebo-controlled trial is considered the most robust of clinical trials, with randomisation and double-blinding enabling minimisation of subject bias and observer bias (Staudacher et al. 2017).

All subjects visited QMU to be screened and their eligibility checked. Eligible subjects were required to attend two study mornings (approximately 2 ½ hours each session) six weeks apart. If logistically possible, subjects would attend sessions on the same day of the week as to reduce the influence of lifestyle variances.

The study aimed to assess the impact of consuming β -glucan-enriched oatcakes, delivering 4.46 g of β -glucan to the diet per day, on energy intakes, body composition and markers of metabolic health in healthy overweight-obese adults.

8.1.1 Eligibility of subjects

This study was open to healthy men and woman aged 18-50 years old, who were abdominally overweight/obese, defined by WC measurements of > 94 cm and > 80 cm, respectively, or had a BMI ≥ 30 kg/m². It was essential that subjects were able to provide informed consent to participating in the study and agreed to follow all pre-study requisites, such as abstaining from strenuous exercise, consuming alcohol and caffeine containing drinks 24 hours prior to study mornings, in addition to undergoing 10 hours of fasting. Given the nature of the study, it was also important that subjects did not show signs of dietary restraint, impaired handling of glucose or had hypertension, all of which were measured during screening sessions. Subjects were not permitted to take part in the study if they had CVD or GI disease, or taking medications which may alter appetite, such as levothyroxine. Individuals who were dieting or had food allergies to any of the snack ingredients (wheat, dairy, soya, sesame seeds), were excluded. Postmenopausal, pregnant or breastfeeding females were not eligible. Both snacks were suitable for consumption by vegetarians but not vegans.

8.1.2 Statistical power

In order to have 80% power to detect a difference of 273 kcal or more in daily energy intake over the 6 week period between the two groups, and assuming a SD in energy intake change of around 461 kcal in free-living subjects (estimated from a dietary intervention study by Astbury et al. 2014), using a two-sided test with the level of significance set to 5%, 45 participants were required in each group at the end of the study. Allowing for up to 10% attrition and unusable data (Moroshko et al. 2011), 50 participants were required for enrolment in each group, therefore 100 participants in total for the two groups.

8.1.3 Recruitment

Subjects were recruited to take part in this research study between October 2015 and March 2017 via convenience sampling methods.

The purpose of recruitment was to, i) screen and check the eligibility of subjects, and ii) test an adequate number of eligible subjects to fulfil the sample size estimated to meet the outcomes of this study. There were no monetary incentives offered to subjects, however the researcher did offer nutritional feedback from food diaries and provided subjects with information gathered at the screening session.

Subjects were screened and enrolled onto the study between October 2015 and February 2017. Subjects did not take part in the study between December 2015 – January 2016, and December 2016 – January 2017 to avoid potential confounding factors, such as increased calorie consumption and altered eating patterns associated with the festive period.

The researcher initially made informal enquiries by telephone and email to local community centres and groups in and around the Musselburgh area. When positive feedback was received from these locations, posters and leaflets were distributed. All recruitment material were approved by Queen Margaret University Research Ethics Panel. Material included information regarding the study that was suitable for the lay public, and contained the researcher's contact details (address, email and office telephone number). In addition to posters and flyers, an advertisement was also circulated throughout Queen Margaret University's email system to all staff and students periodically for approximately 18 months. During the summer months, responses from students and local areas diminished. In order to increase interest and find more subjects, a press release was posted on the Queen Margaret University website and social media pages. After the researcher received notice of interest, contact details of potential subjects were recorded in a password protected Excel file. This ensured all subjects were contacted and followed up in a timely manner, which was at times challenging when high numbers of responses were received, i.e., following the press release.

In order to successfully complete the study, eligible subjects were required to attend the screening session (up to 60 minutes in duration) and two study mornings (around 2 ½ hours each session). Subjects were asked to avoid making any unnecessary changes to their exercise regime and habitual diet, the only exception of which was to incorporate the study snack into their diet daily for six weeks. Subjects were not permitted to go on holiday during the study period.

8.2 Screening session

Interested individuals attended a consultation session with the researcher to discuss eligibility for participation, followed by specific screening tests which determined their suitability. If the subject was found to meet the inclusion criteria, they were given the participant information sheet (**Appendix 7**) to read over again and given time to ask relevant questions. Before any screening tests were performed, the subject gave signed consent.

To determine if potential subjects met the full study criteria, an informal screening interview was performed. A series of questions were asked with regards to:

- Date of birth
- Smoking status
- History of metabolic/ CVD or GI disease
- Allergies or food intolerances
- Medications or supplements
- Snacking habits
- Weight stable in the past 3 months?

Anthropometry was then measured in order to determine BMI and WC (described in full, **section 8.7**). A single use lancet (Accu-Chek Safe T Pro Plus, Roche Diagnostics, UK) was used to take a finger-prick

sample of blood to check fasting blood glucose. Subjects with a fasting blood glucose level of > 5.6 mmol/L were not eligible to take part (as per ADA criteria for impaired glucose control; ADA 2016).

Dietary restraint refers to the tendency to restrict food intake in order to control bodyweight. It was not realistic to include subjects in this study who demonstrated this behaviour, as research outcomes could be biased due to a conscious decision to limit energy intake, which may also be followed by a period of binge eating. Subjects were provided with a DEBQ (**Appendix 3**) in order to measure their dietary constraint. A threshold score of ≤ 2.97 was classed as normal for subjects with BMI ≥ 26 (van Strien et al. 1986).

A physical activity questionnaire (SPAQ; Lowther et al. 1999) was administered during the screening session to allow the researcher to determine physical activity levels of all subjects (**Appendix 4**). A baseline 7-day food diary was given to the subject to complete before returning to the first study session.

8.3 Study morning protocol

On each study day subjects were required to arrive at QMU following an overnight fast of 10 hours; testing was carried out during mornings only. Subjects were advised to wear light clothing on both study sessions and, if possible, wear short sleeves to gain easy access to the upper arm for BP measurements. Subjects were required to avoid strenuous exercise 24 hours prior to the study, and abstain from alcohol and caffeine consumption. An email reminder was sent 48 hours prior to the study session to each subject to reiterate study instructions.

On arrival to the research room, subjects were asked to remove any outdoor clothes and instructed to have five minutes of seated rest before BP measurements were taken. Subjects then removed their shoes and any objects from their pockets and had their height and weight taken. A finger-prick sample of blood was taken to determine fasted blood glucose before 410 mL of Lucozade Original juice was given to the subject to drink. The subject was required to drink the glucose load within five minutes. Immediately after the subject consumed all of the glucose load, a timer was set to alert the researcher of the next glucose sample to be taken 30 minutes later. Blood glucose was measured across five time-points over the morning; fasted, 30,

60, 90 and 120 minutes following glucose load ingestion. In between blood glucose measurements, the researcher measured body composition by dual frequency BIA, in addition to WC and SAD using validated protocols. **Figure 17** summarises measurements taken over the course of each study session. The researcher also administered a physical activity questionnaire for the subject to complete during the session. The subject was required to give a 7-day recall of all physical activities, including walking and strenuous exercise. At the end of the study session, the subject was issued with two 7-day food diaries and their study snacks. During weeks three and six of the study, subjects were required to complete 7-day food diaries. In order to encourage study compliance, the researcher informed the subject that they would receive telephone calls and/or emails to give study reminders.

Subjects returned after six weeks, where all anthropometric measurements, BP and oral glucose tolerance test (OGTT) were re-measured. Subjects completed a second physical activity questionnaire and provided sensory feedback regarding their test snack.

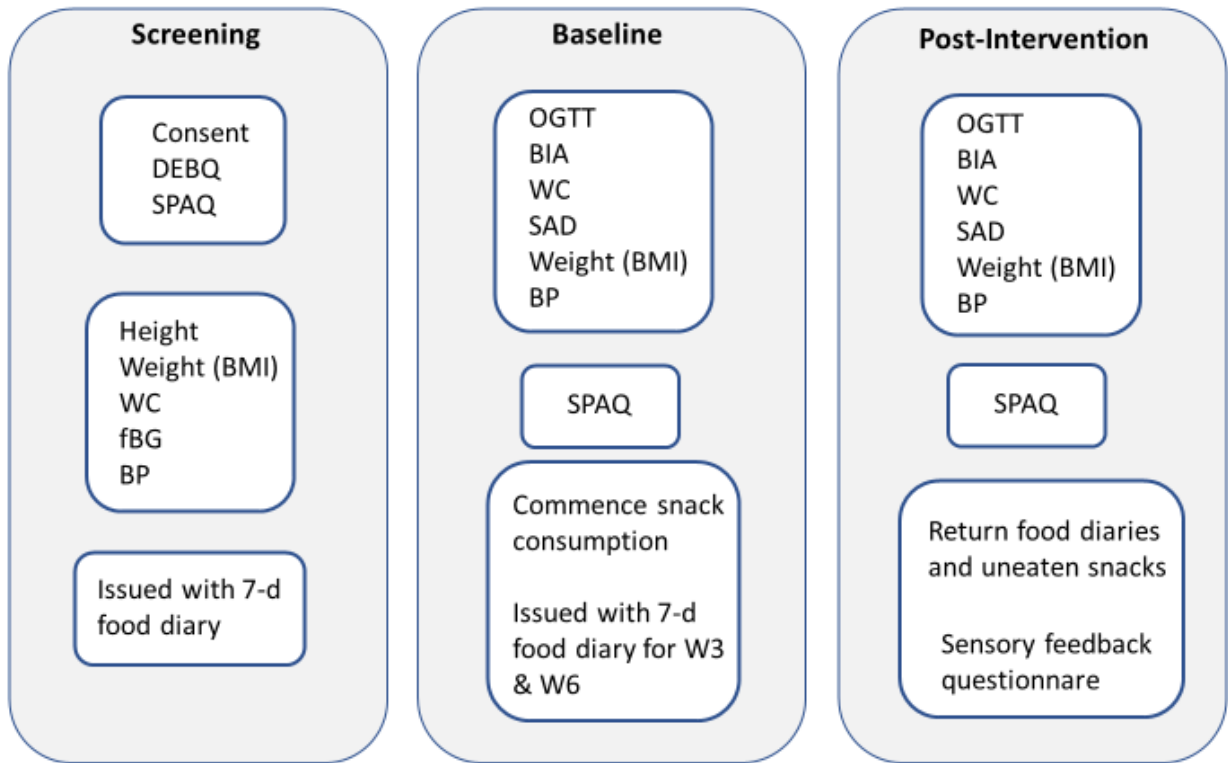


Figure 17. Summary of study B measurements taken over the course of the study period.

BIA, bioelectrical impedance analysis; BMI, body mass index; BP, blood pressure; DEBQ, Dutch Eating Behaviour Questionnaire; f BG, fasted blood glucose; OGTT, oral glucose tolerance test; SAD, sagittal abdominal diameter; SPAQ, Scottish Physical Activity Questionnaire; W3, week-three; W6, week-six; WC, waist circumference; 7-d, 7-day food diary

8.4 Study snacks

8.4.1 Manufacturing β -glucan-enriched oatcake

A raw mix of β -glucan-enriched oatcakes was prepared in the proportions as described in **Table 13**. The ingredients were calculated to provide a minimum dose of 4 g β -glucan in the finished product. High-MW PromOat® (Tate & Lyle, London, UK) oat bran powder was incorporated into a traditional Nairn's rough oatcake recipe.

Table 13. Recipe for β -glucan-enriched oatcakes per pack (5 oatcakes)

Ingredient	Weight (g)
Nairn's oatmeal	228.5
Palm oil	16.5
High oleic sunflower oil	18.75
Sodium bicarbonate	0.52
Salt	4.7
PromOat (35%)	62.5
Water	220

A 552 g mix was made as per recipe, with the addition of 220 g water added and mixed for approximately five minutes at a slow speed, 120 rpm, in a V5 TEDDY commercial mixer (Varimixer, Broendby, Denmark). A further 150 g of water was added, and mixed well. PromOat® was then added and mixed for two to three minutes until the consistency resembled breadcrumbs. Finally, 70 g of water was poured into the mixing bowl whilst running and mixed for one to two minutes. The resulting dough was then processed through the lab brake. Dough was cut using a 66 mm cutter, with a height of 2 mm. Once cut, the dough pieces were placed onto an aluminum tray and baked in the lab oven at 180°C for 14 minutes. **Figure 18 (panel a)** shows the final product. Baked oatcakes were inserted into a biscuit packing system (flow wrapper, Bosch, Robert Bosch Packaging Technology GmbH, Germany) and sealed in pouches (**Figure 18, panel b**). Preparation, baking and packaging of oatcakes were carried out by a food technician at Nairn's Oatcakes Ltd., Peffermill, Edinburgh.

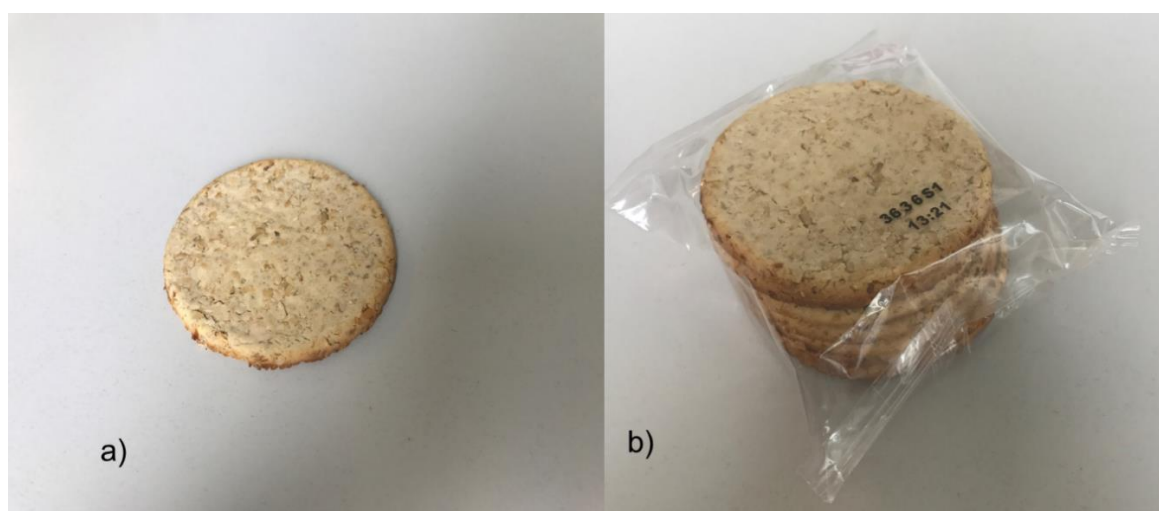


Figure 18. β -glucan-enriched oatcakes manufactured by Nairn's Oatcakes Ltd. a) baked single oatcake, ready to eat. b) packed and sealed finished product

8.4.2 Determination of β -glucan content and nutritional analysis

As a means of quality control, and to ensure that the manufacturing process did not reduce the β -glucan content, the researcher collaborated with Dr. Gordon McDougall (The James Hutton Institute, Dundee) to quantify β -glucan contents of the test snacks. β -glucan content was determined using a β -Glucan Assay Kit (Mixed Linkage) manufactured by Megazyme, Leinster, Ireland. This assay is specific for (1 \rightarrow 3,1 \rightarrow 4)- β -D-glucan from cereal sources and has a number of certifications from various organizations, including the American Association of Cereal Chemists (AACC) and American Chemical Society (ACM) (Megazyme 2015). Full nutritional analysis of the β -glucan-enriched oatcakes was performed in an accredited lab by ALS Food and Pharmaceutical. Samples of oatcakes from all batches manufactured by Nairn's were nutritionally tested and β -glucan content quantified. Energy, macronutrient and β -glucan content per 100 g are shown in **Table 14**.

Table 14. Macronutrient composition of β -glucan-enriched oatcakes per 100g

	Batch 1	Batch 2	Average
Energy (kcal)	425	427	426
Protein (g)	9.14	8.55	8.85
Carbohydrate (g)	66.7	67.7	67.2
Available CHO (g)	50.3	51.0	50.3
Sugar (g)	1.4	1.4	1.4
Fibre (g)	16.4	16.7	16.6
<i>of which β-glucan (g)</i>	8.3	8.3	8.3
Total fat (g)	17.2	17.3	17.3
Saturated fat (g)	3.96	3.82	3.89
Monounsaturated fat (g)	9.13	9.18	9.15
Polyunsaturated fat (g)	3.3	3.5	3.4
Trans fat (g)	0.05	0.05	0.05
Sodium (mg)	577	599	588

8.4.3 Test snacks

After the final oatcake recipe and nutritional analysis were determined, a suitable control snack was identified. The natural β -glucan content of oats ruled out the option to use an alternative oatcake as a control snack; idealistically a suitable control snack should not contain β -glucan. McVities Krackawheat crackers (Jacob's, Leicestershire, UK) were identified as a suitable savoury snack, thus an appropriate portion size was calculated to match the caloric and macronutrient content of the intervention snack (**Table 15**).

Subjects were randomly assigned to either the control or intervention group, receiving 48.1 g (6 ½ crackers) or 53.7 g (5 oatcakes) per day, respectively. Randomization was performed using a random number generator by a researcher impartial to the study. In order for the study to be double-blinded in nature, the researcher was assisted by a PhD student, who was impartial to the study. Test snacks were

given to the subject ‘double-bagged’ to conceal the snacks from the researcher. All test snacks (six week supply) were issued at end of the baseline session and subjects were instructed not to remove test snacks from the bags until they left the research room. Test snacks were issued in sealed pouches for convenience and to avoid the snacks drying out. Subjects were required to consume one pouch of snacks per day, for six weeks, which could be consumed throughout the day. Subjects were instructed that the study snack should not be shared with others. Study test snacks were logged in food diaries as “Study snacks” to avoid bias when nutritional analysis was performed by the researcher. Subjects were asked to return uneaten snacks at their final session in order to monitor study compliance.

Table 15. Nutritional composition of control (Krackawheat) and intervention (β -glucan oatcakes) snacks consumed daily for six weeks

	Krackawheat	β-glucan
Daily serving (g)	48.1g	oatcakes
		53.7g
Energy (kcal)	221	229
Protein (g)	4.55	4.75
Carbohydrate (g)	29	27
Sugar (g)	1.3	0.8
Fibre (g)	2.6	8.8
<i>of which β-glucan (g)</i>	0	4.46
Total fat (g)	9.1	9.4
Saturated fat (g)	4.6	2
Sodium (mg)	0.65	0.32

8.5 Energy intakes

Subjects were issued with an estimated 7-day food diary prior to commencing the study in order to determine baseline energy intake. In order to ensure greater accuracy in the recording of consumed quantities of food, information to help estimate portion sizes was provided (BDA 2016). The researcher verbally explained to the subject the importance of documenting as much information as possible in their food diaries. Subjects were encouraged to keep food packaging and labels if they were unsure how to log certain foods/meals, which could be returned to the researcher along with the food diary. Diet diaries were returned to the researcher in person at a following testing session or else returned via post, where a self-addressed, franked envelope was supplied (especially in the case of the last testing day). During study sessions, the researcher checked the diet diaries for completeness and asked any relevant questions to clarify food/meal descriptions. Follow up emails were made to clarify any unclear information such as food brands and portion sizes when necessary.

A 'symptoms' table was included in the food diaries completed during weeks three and six. This gave the subject the opportunity to detail any changes in bowel movements, bloating, nausea etc. that may be associated with their study snack. All food diaries were analysed after completion of the study to avoid un-blinding the researcher. Total energy intake, macronutrients and fibre intake were calculated using Nutritics® nutritional analysis software (version 4.0, Nutritics Ltd., Dublin, Ireland).

8.5.1 Displacement of Energy and Nutrients

Intakes of total energy, macro- and micronutrients were compared and nutrient displacement was calculated using the method of Kranz et al. (2013). According to this method, energy and nutrient intake levels as a result of the addition of snacks to the diet, referred to here as the calculated snack diet (C), are equal to the energy and nutrient intake during the usual diet (U) plus the energy, macro- and micronutrients provided by the snacks ($C = U + \text{snacks}$). Displacement (D) of energy and nutrients in absolute terms was determined by subtracting the intake of energy and nutrients when on the actual snacking periods (A3 and A6) from the calculated amount (C) ($D = C - A$).

8.5.2 Underreporting

As previously mentioned, underreporting (and overreporting to a lesser extent) of energy intakes is the predominant drawback to utilising estimated food diaries in energy balance research. Underreporting was potentially problematic for the current study as the primary outcome measured was energy intake. Therefore, it was important to evaluate the magnitude of underreporting in the current study to reduce the potential bias in energy intake estimates. The most common method is the Goldberg cut-off method for identifying under- and overreporters within study groups.

The principles of the Goldberg cut-off method and the statistical derivation of the equations to calculate them were described originally by Goldberg and colleagues (Goldberg et al. 1991). More recently, the equations have been restated and the factors to be used are revised by Black (Black 2000a; Black 2000b). Information on age and gender of each subject, individual energy intake, weight, height and physical activity data were required to implement the Black (Black 2000a) method. Specific lower cut-off and upper cut-off values for subject groups to identify under- and over-reporters were identified using the following formulas (extrapolated from EFSA 2013):

Lower cut-off $E_{rep}: BMR_{est} > PAL \times \exp [SD_{min} \times ((S/100)/\sqrt{N})]$

Upper cut-off $E_{rep}: BMR_{est} < PAL \times \exp [SD_{max} \times ((S/100)/\sqrt{N})]$, where

SD_{min} is -2 for the 95% lower confidence limit, SD_{max} is +2 for the 95% upper confidence limit, N was the number of subjects included in each defined group. Reported Energy Intake (E_{rep}) was calculated as average value on the basis of energy intakes. Estimation of BMR (BMR_{est}) of each studied subject was performed applying the equations of Schofield (1985; **Table 16**). **Appendix 8** details the steps followed to calculate over- and underreporting in the current study.

Table 16. Schofield equations for estimating BMR (kcal/d) from weight (kg) and height (m)

Gender/Age (years)	BMR (kcal/day)
Male	
18-29	$15.0 \times \text{weight} - 10 \times \text{height} + 706$
30-59	$11.5 \times \text{weight} - 2.6 \times \text{height} + 877$
Female	
18-29	$13.6 \times \text{weight} + 283 \times \text{height} + 98$
30-59	$8.1 \times \text{weight} + 1.4 \times \text{height} + 844$

8.6 Body composition

A Bodystat®1500 MDD dual frequency body composition and wellness monitoring unit (Bodystat Ltd., Isle of Man, UK) was used to determine body fat percentage and estimated lean muscle mass in all subjects throughout the duration of the study.

Subjects were instructed to remove their right shoe and sock before laying down on a clinical plinth (non-conductive surface). Once the subject was lying flat, it was explained that no parts of their body should touch one another in order to give an accurate reading. For subjects who were considerably overweight, their arms and inner thighs often made contact with their body despite moving them apart slightly. These subjects were encouraged to find a comfortable position at the same time as spreading their arms and legs fully before the test was initiated. Self-adhesive disposable electrodes were attached to the right hand and right foot of the subject in order to avoid the battery current (low voltage) passing through the side of their body where their heart is situated. Two electrodes were positioned on the right hand; one behind the knuckle of the middle finger, and the second on the wrist next to the ulna head. Two electrodes were also placed on the right foot; one behind the second toe next to the big toe, and the second on the ankle at the level between the medial and lateral malleoli (the large protruding bones on the side of the ankle). The leads were then attached to the tab strips on the electrodes. The red leads were connected to the electrodes located behind the finger and toe, whereas the black leads were connected to the electrodes on the wrist and ankle. The positioning of both electrodes and leads is shown

in **Figure 19**. Once the subject was in position, they were encouraged to relax for at least 3-4 minutes before the test was run, allowing time for fluid levels to stabilize in the body. Subject information was then entered into the Bodystat device (gender, age, height, weight, activity level) before running the test. In order to determine the appropriate activity level for each subject, the researcher evaluated the level of physical activity for each subject based on their responses reported in the SPAQ. The activity level determined for the subject at the baseline session was also used for post-study BIA.



Figure 19. Correct positioning of electrodes and lead attachment for BIA testing

8.7 Anthropometry

8.7.1 Anthropometric Technical Error of Measurement (TEM)

When anthropometrical measures are reported, variability of the measures may occur as a result of the physical diversity of the cohort analysed, biological variation (that cannot be avoided) or due to technical variations (that can be avoided). It is important for the researcher to be aware of measurement accuracy, particularly when it is anticipated that changes in body composition may be seen during such research (Franco-Villoria et al. 2016). The International Society for the Advancement of Kinanthropometry (ISAK 2001) advocates that technical error of measurement (TEM) is calculated. The researcher used the following equation to determine TEM:

Absolute TEM = $\sqrt{\left(\frac{\sum di^2}{2N}\right)}$, where $\sum d^2$ is the summations of deviations raised to the second power, N is number of volunteers measured, and i is the number of deviations.

Furthermore, the absolute TEM was transformed to relative TEM in order to obtain the error expressed as a percentage, which corresponded to the total average of the variable to be analysed:

Relative TEM = $\frac{TEM}{VAV} \times 100$, where VAV is the variable average value (Perini et al. 2005).

The researcher calculated relative TEM for height, WC and SAD in 10 departmental colleagues before commencing data collection: height = 0.03 cm (2%), WC = 0.08 cm (9%) and SAD = 0.04 cm (20%).

8.7.2 Sagittal abdominal diameter

The subject was asked to assume a relaxed standing position with their arms folded across their chest, breathing normally. The standing position is a valid method for measurement of SAD to negate the possible excessive contribution of visceral fat measured using a supine position (Iribarren et al. 2006). The researcher stood to the side of the subject and extended the large sliding calipers (Rosscraft, Surrey, Canada). In order to avoid restricting ease of movement, the researcher held the calipers with her thumb on the top of the caliper blade, not between the blades. The edge of one caliper blade was placed on the anterior skin surface immediately inferior to the omphalion at the most anterior protuberance. The other caliper was slowly closed until it met the skin surface superficial to the lumbar spine. The normal breathing pattern of the subject was followed for at least two cycles, with the measurement taken at end-tidal expiration. Care was taken not to leave indentations on the skin surface. Subjects were encouraged to relax and to avoid contraction of their abdominal muscles. If however, the level of the umbilicus was lower than the 5th lumbar spinal process, then the measurement was taken starting from the base of the lumbar spinous process presenting the greatest curvature to the corresponding point of the abdomen. The full protocol has been presented previously by Marfell-Jones et al. (2012).

8.7.3 Waist circumference

Subjects assumed a relaxed standing position with their arms folded across their thorax with their feet approximately 30 cm apart. The measurement was taken at the narrowest point between the lower costal

border (10th rib) and iliac crest. If there was no obvious narrowing, the measurement was taken at the midpoint between these two landmarks. The subject was instructed to lower their arms to a relaxed position, with the tape being readjusted if required. The researcher followed the normal breathing pattern of the subject and took the measurement at the end of their normal expiration. All WC measurements were taken in accordance to ISAK (2001) protocol, which is the gold standard for measuring abdominal body girth.

8.7.4 Height, Weight and Body Mass Index

Subjects were required to wear light clothing and no shoes. Body weight was measured to the nearest 0.1 kg using an automated calibrated electronic scale (Salter 90185S SV3R, Kent, UK). Before subjects were asked to stand on the scales they removed all items from their pockets, if necessary. Standing height was measured without shoes to the nearest 0.1 cm using a fixed wall stadiometer (Seca 206, Birmingham, UK). Subjects stood erect on the floor with their back to the stadiometer, their arms hanging by their sides with their weight evenly distributed on both feet. The horizontal bar was lowered to make contact with the crown of the subject's head with sufficient pressure to compress the hair. The researcher ensured the subject's head was positioned in the Frankfort Horizontal Plane position. The Frankfort Plane is an imaginary line passing through the external ear canal and across the top of the lower bone of the eye socket, immediately under the eye (ISAK 2001). Accessories worn in the hair were removed from the subject in order to obtain an accurate measurement. Weight was coupled with height to calculate BMI as per the following equation (WHO 2000): $BMI = kg / m^2$.

8.8 Blood pressure

The measurement protocol has a significant role in the accuracy of the BP results. Resting time before measurements, posture of the subject and placement of the cuff are pivotal to standardizing the procedure (Tolonen et al. 2015). An Omron®M5-1 digital monitor (Omron, Milton Keynes, UK) was used to record BP readings in triplicate and in accordance with the protocol suggested by Society of Hypertension Working Group on Blood Pressure Monitoring (O'Brien et al. 2003). The participant was seated for at least five minutes without moving or speaking and was encouraged to relax before

measurements were taken. The participant was instructed not to cross their legs in order to avoid interference with peripheral blood flow. Tight clothing that could potentially cause restriction to the arm was removed and the arm of which wore the BP cuff was supported at the level of the heart. The cuff was placed neatly around the arm with the indicator mark on the cuff over the brachial artery. The researcher ensured that the cuff did not encircle more than 80% of the arm. The participant was told when the cuff would be inflated in order to reduce possible anxiety. Systolic blood pressure, DBP and heart rate were displayed on screen and noted down by the researcher. The first BP measurement was taken in both arms, with subsequent measurements taken with the arm which gave the highest reading. Blood pressure measurements were taken three times, with 5 minutes of rest between measurements.

8.9 Oral Glucose Tolerance Test (OGTT)

Finger-stick methods have been proven as accurate as commercial laboratory methods, regardless of blood collection methods (McLoughlin et al. 1994; Olansky and Kennedy 2010). Participants arrived at the research centre having fasted since 22:00 the previous evening (fast \geq 10 hours). An alcotip pre-injection swab (Universal™ 70% isopropyl alcohol, Oxford, UK) was used to clean the puncture site before a fasting blood sample was collected from the fingertip using a single use lancet (Accu-Chek Safe T Pro Plus, Roche Diagnostics, UK). The subject was given 410 mLs of Lucozade© (Lucozade Ribena Suntory Ltd., UK) from a standard Lucozade© bottle (70 kcals/100 ml), which was the equivalent to 75 g anhydrous glucose (NHS 2000). Subjects were instructed to consume the glucose load within 5 minutes. A second fingerprick blood sample was taken 30 minutes after the subject had consumed the glucose load and again at 60, 90 and 120 minutes after. Measurements of glucose were determined using Accu-Chek® Aviva monitor (Roche Diagnostics, UK) test-strips and monitor.

8.10 Sensory feedback

All subjects completed a sensory feedback questionnaire during their final study session. Subjects were asked to comment on visual appeal, smell, taste, aftertaste and palatability of their test snack, and were encouraged to give as much information as possible with regards to ease of consumption and boredom with taste (**Appendix 9**).

8.11 Statistical analysis

The primary outcome of this study was energy intakes, with body weight, WC, SAD, body fat %, BMI, along with markers of metabolic health, glucose tolerance and BP, as secondary outcomes.

Data was analysed using SPSS for windows (version 23.0, SPSS Inc. Chicago, IL).

A Shapiro-Wilk Test of Normality was performed in order to determine the distribution of the data collected. Shapiro-Wilk is the appropriate normality test for small sample sizes ($n < 50$). Values are reported as means with SEM, unless otherwise stated. A p value of < 0.05 was considered significant.

Total energy intake (kcal) and macronutrient intakes were compared across three periods, baseline and during weeks three and six of the study. Student's independent t-tests were carried out to determine differences between the control snack group and β -glucan snack group at baseline, week 3 and week 6 for energy and nutrient intakes, including displacements. To assess changes within groups, one-way analysis of variance (ANOVA) was performed to identify statistical changes. Where ANOVA detected significant differences, *post hoc* Bonferroni analysis was performed in order to identify between which weeks caloric intakes differed significantly.

With regards to secondary outcome measures, total AUC for glucose was calculated using the trapezoidal method. For glucose AUC, BP and all anthropometric measurements, Student's independent t-tests were performed to compare groups. In order to identify differences within study snack groups (baseline vs. week 6), Student's paired t-tests were carried out.

Chapter 9

9.0 Effects of a six-week intervention with novel β -glucan-enriched oatcake snacks on daily energy intakes, body composition and markers of metabolic health in overweight and obese individuals: a pilot study (Study B): Results

9.0.1 Recruitment

The flow of participants through the study, from recruitment to study completion and analysis, can be seen in **Figure 20**. Fifty-nine individuals responded to the recruitment material and contacted the researcher to express interest in study participation. Of the 59 responses, 25 individuals attended the screening session at Queen Margaret University. Reasons for not attending the screening session included: no response, n=18; time constraints n=5; GI intolerances, n=3; smoker, n=5; other, n=3. Twenty-three individuals met the inclusion criteria and enrolled onto the study. Two individuals were excluded from participation; one individual had a fasted blood glucose measurement >5.6 mmol/L and took antidepressant medication, and one subject had an intolerance to gluten. Two subjects withdrew from the study before attending the first study morning; both subjects had work commitments and could no longer participate. A total of 21 subjects completed the study. There were no subject drop outs during the intervention period.

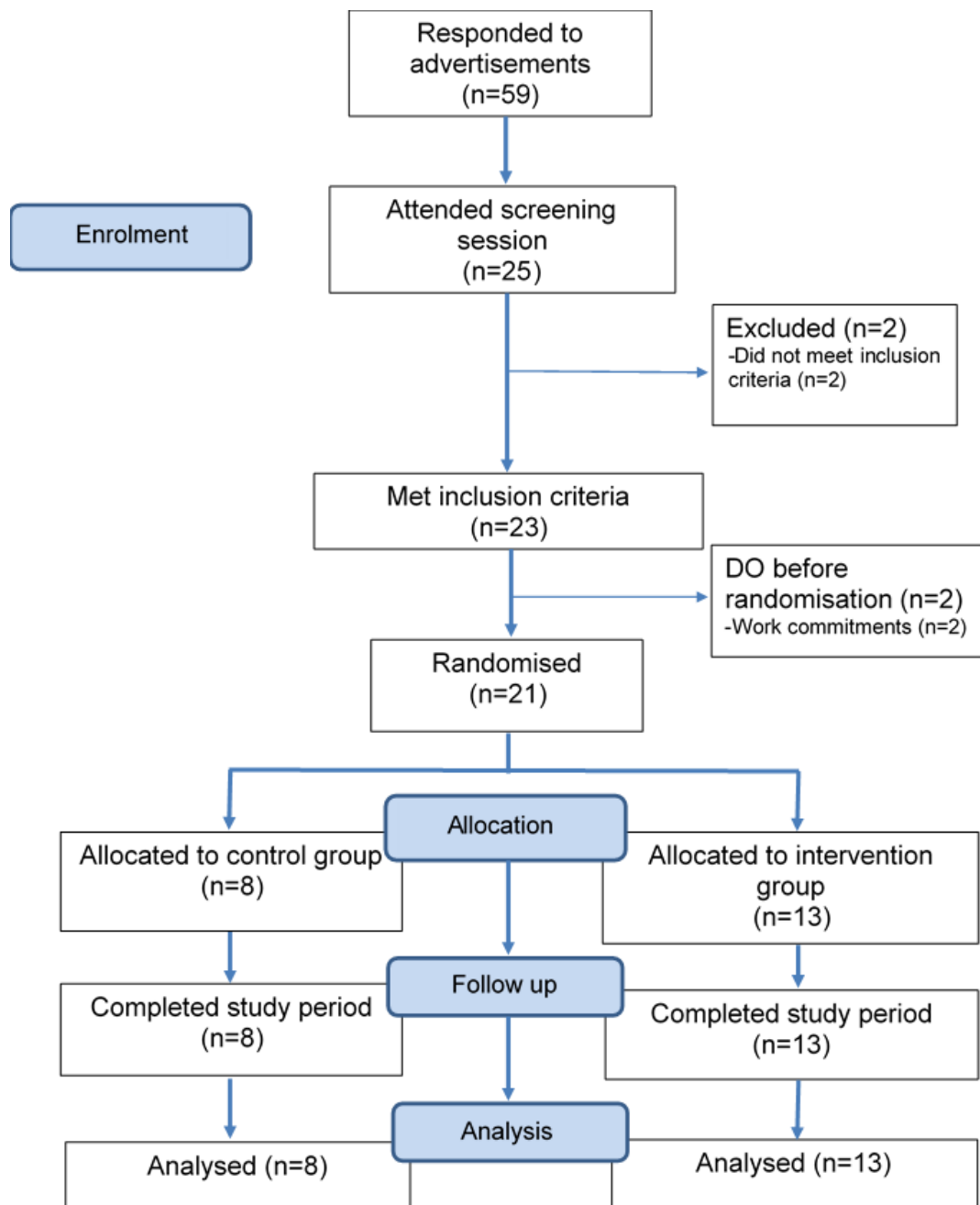


Figure 20. Overview of subjects from recruitment to study completion. *DO* drop out.

9.0.2 Subjects Characteristics

All subjects were healthy, with no previous history of metabolic or CVD or took medications which may affect appetite. None of the subjects were smokers, were under a calorie-restricted diet and all reported to be weight stable. None of the subjects had food allergies to test snack ingredients. A mean score of <2.97 was indicative of no dietary restraint in study subjects who had a mean BMI >26 kg/m² (van Strien et al. 1986). **Table 17** summarises subject characteristics from information collated during screening sessions.

Table 17. Subjects' characteristics at screening visit

	Control (n=8)	β-glucan (n=13)	p value
Sex	6 female / 2 male	11 female / 2 male	0.31
Age (years)	37 ± 11	34 ± 9	0.53
Height (m)	1.7 ± 0.1	1.7 ± 0.1	0.56
Weight (kg)	88.0 ± 12.0	86.5 ± 20.3	0.86
BMI (kg/m ²)	29.7 ± 3.5 (25.1-33.3)	29.8 ± 4.4 (26.1-39.1)	0.98
WC	100.9 ± 7.9	95.0 ± 11.4	0.21
Physical activity (min/week)	556 ± 203	1109 ± 754	0.025
Restraint eating score	2.0 ± 0.5	2.2 ± 0.6	0.56
Fasted blood glucose (mmol/L)	4.8 ± 0.4	4.8 ± 0.5	0.97

Data are means ± SEM, numbers in brackets indicate range.

9.0.3 Adverse effects

From the control group, only one subject reported mild flatulence and mild bloating during week 6 of the study.

From the intervention group, increased flatulence was reported in six subjects during week 3 and week 6. Bloating was also reported during week 3 and 6 in six subjects. Five subjects reported changes in their stools. With regards to written feedback regarding GI changes, one subject reported that, “*the oatcakes influenced [his] bowel habits positively (consistency).*” **Table 18** details the severity and frequency of reported GI side effects reported during the study period during test snack consumption.

Table 18. Gastrointestinal side effects reported in food diaries following snack consumption for 3 and 6 weeks.

	Flatulence			Bloating			Changes in stool		
	mild	moderate	a lot	mild	moderate	severe	mild	moderate	severe
Week 3									
Cracker									
β-glucan	1	2	1	3	2	1	3	1	1
Week 6									
Cracker	1			1					
β-glucan	2	1	1	3	2	1	3	1	1

Data collated from all subjects who returned food diaries, control cracker group, n=6; β-glucan-enriched oatcake group, n=11.

9.1 Energy intake

Not all subjects returned the 7-day food diary data. Subjects who returned a baseline and either week 3 or week 6 food diary were included in the energy intake analysis. Subjects who failed to return their baseline food diary were not included in energy intake analysis. In the control group, one subject failed to return all food diaries and one subject did not return their week 6 food diary. In the intervention group, two subjects failed to return their baseline food diaries.

Evaluation of food diaries at mid-way (week 3) through the study and at the end (week 6) indicated little variation between groups for energy and macronutrient intakes at each time point with no significant difference differences between groups.

The total energy and nutrient intakes at baseline, week 3 and week 6 for each group are presented in **Table 19**. There were no statistical differences identified in energy intakes throughout the study between groups; baseline ($p=0.23$), week 3 ($p=0.39$), week 6 ($p=0.58$). There were no changes in energy intake within the control and β -glucan groups (ANOVA one-way; $p=0.74$, $p=0.42$, respectively).

There was a statistically significant difference reported between groups for dietary saturated fatty acid (SFA) intake during week 3 of the study period. Mean SFA intakes in the intervention group were significantly lower than control group intakes, 26.2 ± 2.8 g vs. 38.5 ± 5.5 g, respectively, ($t(16)=2.15$, $p=0.047$).

Trans-fatty acid (TFA) intakes were also significantly lower in the intervention group compared to control group during week 3, 0.7 ± 0.1 g vs. 1.3 ± 0.3 g, respectively, ($t(16)=2.0$, $p=0.043$).

There were no other significant changes for macronutrients between groups; protein, total fat, including MUFA, and PUFA, CHO, including sugars, or dietary fibre or starch (all $p>0.05$ for week 3 and week 6).

No differences were reported between groups for micronutrients; sodium, potassium or calcium (all $p>0.05$ for week 3 and week 6).

Water or alcohol intake were not statistically different between groups (all $p>0.05$ for week 3 and week 6).

Table 19. Total energy and nutrient intakes at baseline and during the intervention for both groups

	Baseline		Week 3		Week 6	
	Control n=7	β-glucan n=11	Control n=7	β-glucan n=11	Control n=6 ^A	β-glucan n=11
Energy (kcal)	2453 ± 316	2018 ± 197	2226 ± 238	1973 ± 170	2140 ± 293	1987 ± 121
kJ	10262 ± 1323	8445 ± 824	8389 ± 996	7296 ± 713	8030 ± 1226	7355 ± 508
Protein (g)	114 ± 15	83 ± 11	107 ± 14	75 ± 8	98 ± 13	76 ± 7
% of TE	19 ± 2	16 ± 1	19 ± 1	15 ± 1	19 ± 2	15 ± 1
Total fat	102 ± 17	86 ± 11	102 ± 15	83 ± 9	85 ± 11	83 ± 7
% of TE	37 ± 3	38 ± 2	41 ± 3	38 ± 2	36 ± 3	37 ± 2
MUFA (g)	29.7 ± 6.0	29.2 ± 4.0	32.0 ± 5.2	26.7 ± 3.5	25.1 ± 3.9	24.7 ± 2.6
% of TE	10.5 ± 1.1	13.2 ± 1.4	12.8 ± 1.2	12.0 ± 0.8	10.8 ± 1.4	11.1 ± 0.7
PUFA (g)	13.7 ± 2.4	13.2 ± 1.7	14.0 ± 2.5	12.1 ± 1.5	9.5 ± 2.3	10.7 ± 1.2
% of TE	5.2 ± 0.9	5.9 ± 0.6	5.5 ± 0.5	5.4 ± 0.5	3.9 ± 0.5	4.7 ± 0.4
SFA (g)	37.4 ± 7.1	30.7 ± 4.7	38.3 ± 5.5	26.2 ± 2.8 *	33.3 ± 3.6	27.2 ± 2.7
% of TE	13.3 ± 1.3	13.2 ± 0.8	15.3 ± 0.8	11.9 ± 0.8 **	14.6 ± 1.5	12.1 ± 0.7
TFA (g)	1.1 ± 0.2	1.2 ± 0.3	1.3 ± 0.3	0.7 ± 0.1 #	1.0 ± 0.2	0.9 ± 0.2
% of TE	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1

<i>Continued</i>	Baseline		Week 3		Week 6	
	Control	β-glucan	Control	β-glucan	Control	β-glucan
CHO (g)	244 ± 39	203 ± 20	204 ± 19	200 ± 17	212 ± 33	204 ± 11
% of TE	41 ± 5	41 ± 3	38 ± 3	41 ± 2	40 ± 5	42 ± 2
Sugar (g)	123 ± 39	86 ± 9	78 ± 14	71 ± 9	94 ± 21	73 ± 8
% of TE	19 ± 5	17 ± 1	15 ± 3	14 ± 1	18 ± 4	15 ± 1
Dietary fibre (g)	23 ± 2	20 ± 3	24 ± 3	26 ± 2	21 ± 4	26 ± 2
Starch (g)	119 ± 7	115 ± 14	96 ± 16	101 ± 9	86 ± 18	103 ± 9
Cholesterol (mg)	317 ± 84	273 ± 48	282 ± 63	213 ± 24	240 ± 56	229 ± 32
Sodium (mg)	3268 ± 539	2206 ± 227	2528 ± 386	1947 ± 152	2442 ± 333	1975 ± 206
Potassium (mg)	3165 ± 430	2683 ± 370	2934 ± 465	2591 ± 232	2950 ± 523	2351 ± 231
Calcium (mg)	791 ± 83	689 ± 57	654 ± 96	571 ± 98	742 ± 102	709 ± 134
Water (mL)	2204 ± 107	2089 ± 200	1663 ± 248	2092 ± 236	2008 ± 227	2109 ± 241
Alcohol (g)	14 ± 8	14 ± 5	8 ± 4	14 ± 4	18 ± 11	14 ± 4
% of TE	3 ± 2	5 ± 1	2 ± 1	5 ± 1	5 ± 2	5 ± 1

^Δ one subject failed to return week 6 food diary. *CHO* carbohydrates, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *SFA* saturated fatty acids, *TE* total energy, *TFA* trans fatty acids. * p=0.047 following independent t-test during week 3 for SFA intakes between control and β-glucan snack group. ** p=0.012 following independent t-test during week 3 for % energy from SFA between control and β-glucan snack groups. # p= 0.043 following independent t-test during week 3 for TFA intakes between control and β-glucan snack groups. All data are means ± SEM.

9.1.1 Energy and nutrient displacement

9.1.1.1 Between snack groups displacement

Overall, there were no significant differences in absolute displacement of energy during weeks 3 and 6 of the study period between the control and intervention group (overall, $p>0.05$).

Similarly, there were no significant findings in absolute displacements in any of the nutrients assessed between groups (all $p>0.05$).

9.1.1.2 Within β -glucan snack group displacement

Table 20 shows total energy (kcal) and selected nutrients of the usual diets (U), the diet calculated with the addition of oatcakes (C), the actual dietary intakes during week three and six of the study (A3, A6, respectively), as well as the contribution from the oatcakes alone and the absolute displacement for week three (D3) and week 6 (D6).

Mean intakes of total energy and nutrients were lower when individuals reported intakes when consuming oatcake snacks (A3, A6) than their usual diet (U). Total energy intake and nutrient intakes were lower during the intervention periods (A3, A6) than the anticipated calculated intakes (C). Significant differences in the intake of nutrients during the snack consumption period, compared to the calculated intakes (C), reflect nutrient displacement.

A displacement of total energy by an average of 500 kcal was seen during week 3 ($t(10)=4.16$, $p<0.01$) and by 487 kcal during week 6 ($t(10)=3.36$, $p<0.01$) of consuming oatcake snacks.

Total dietary fat was also displaced by an average of 21 g during week 3 ($t(10)=4.03$, $p<0.01$) and week 6 by an average of 22 g ($t(10)=3.08$, $p<0.05$), with SFA significantly displaced by an average of 9 g during week 3 only ($t(10)=2.62$, $p=0.02$).

Total CHO was also displaced during week 3 by an average of 57 g ($t(10)=3.93$, $p<0.01$) and week 6 by an average of 53 g ($t(10)=3.61$, $p<0.01$), with sugars significantly displaced by an average of 17 g during week 3 only ($t(10)=3.66$, $p<0.01$).

A displacement was reported in dietary fibre during week 3 by 11 g ($t(10)=6.03$, $p<0.001$) and week 6 by 11 g ($t(10)=4.95$, $p<0.001$).

There were no significant displacements of dietary protein or sodium (overall $p=0.55$, 0.27 , respectively).

Table 20. Dietary intakes and displacement of energy and selected nutrients at week 3 and week 6 of β -glucan-enriched oatcake consumption

	Intake on usual diet (U)	Amount from 53.7g oatcake snack	Calculated intake of oatcake snacking (C)	Actual intake during week 3 of oatcake snacking (A3)	Absolute displacement (D3) week 3	p value (C vs. A3)	Actual intake during week 6 of oatcake snacking (A6)	Absolute displacement (D6) week 6	p value (C vs. A6)
Energy (kcal)	2018 \pm 197	229	2245 \pm 197	1745 \pm 170	500 \pm 184	<0.01	1758 \pm 121	487 \pm 146	<0.01
Protein (g)	83 \pm 11	4.7	87 \pm 11	71 \pm 8	16 \pm 10	0.5	71 \pm 7	16 \pm 8	0.6
Total fat (g)	86 \pm 11	9.39	95 \pm 11	74 \pm 9	21 \pm 10	<0.01	73 \pm 7	22 \pm 7	0.01
SFA (g)	31 \pm 5	2	33 \pm 5	24 \pm 3	9 \pm 4	0.02	25 \pm 3	8 \pm 4	0.07
CHO (g)	203 \pm 20	27	230 \pm 20	173 \pm 17	57 \pm 19	<0.01	177 \pm 11	53 \pm 15	<0.01
of which sugars (g)	86 \pm 9	0.75	87 \pm 9	70 \pm 9	17 \pm 9	<0.01	73 \pm 8	14 \pm 8	0.09
Total fibre (g)	20 \pm 3	8.8	28 \pm 3	17 \pm 2	11 \pm 3	<0.001	17 \pm 2	11 \pm 2	<0.001
Sodium (mg)	2206 \pm 227	0.32	2206 \pm 227	1945 \pm 152	261 \pm 190	0.29	1975 \pm 206	231 \pm 187	0.24

A3, actual intake during week 3 of oatcake snack consumption; A6, actual intake during week 6 of oatcake snack consumption; C, calculated intake of oatcake consumption; CHO, carbohydrate; D3, absolute displacement of calories/nutrient for week 3; D6, absolute displacement of calories/nutrient for week 6; U, intake of usual diet (baseline diet). Data are means \pm SEM. Week 3 data (n=11); week 6 (n=11).

9.1.1.3 Within cracker snack group displacement

Table 21 shows total energy (kcal) and selected nutrients of the usual diets (U), the diet calculated with the addition of control crackers (C), the actual dietary intakes during week three and six of the study (A3, A6, respectively), as well as the contribution from the crackers alone and the absolute displacement for week three (D3) and week 6 (D6). Mean intakes of total energy and nutrients were lower (non-significantly) when individuals reported intakes when consuming control cracker snacks (A3, A6) than their usual diet (U). Total energy intake and nutrient intakes were lower (non-significantly) during the intervention periods (A3, A6) than the anticipated calculated intakes (C).

Dietary protein intakes were significantly displaced by an average of 20 g during week 6 ($t(5)=3.86$, $p=0.01$).

There were no significant displacements in any other nutrients (all, $p>0.05$).

Table 21. Dietary intakes and displacement of energy and selected nutrients at week 3 and week 6 of control cracker consumption

	Intake on usual diet (U)	Amount from 48.1 g cracker snack	Calculated intake of cracker snacking (C)	Actual intake during week 3 of cracker snacking (A3)	Absolute displacement (D3) week 3	p value (C vs. A3)	Actual intake during week 6 of cracker snacking (A6)	Absolute displacement (D6) week 6	p value (C vs. A3)
Energy (kcal)	2453 ± 316	221	2674 ± 316	2226 ± 234	448 ± 282	0.16	2140 ± 293	534 ± 246	0.07
Protein (g)	114 ± 15	4.6	118 ± 15	107 ± 14	11 ± 9	0.27	98 ± 13	20 ± 3	0.01
Total fat (g)	102 ± 17	9.1	111 ± 17	102 ± 15	9 ± 7	0.53	85 ± 11	26 ± 16	0.21
SFA (g)	38 ± 7	4.6	42 ± 7	38 ± 5	4 ± 3	0.61	33 ± 4	9 ± 6	0.34
CHO (g)	244 ± 39	29.3	274 ± 39	204 ± 20	70 ± 39	0.12	212 ± 33	62 ± 36	0.09
of which sugars (g)	123 ± 39	1.3	125 ± 39	78 ± 14	47 ± 30	0.17	94 ± 21	31 ± 29	0.24
Total fibre (g)	23 ± 2	2.6	26 ± 2	24 ± 3	2 ± 1	0.26	21 ± 4	5 ± 3	0.25
Sodium (mg)	3268 ± 539	0.65	3269 ± 539	2528 ± 386	741 ± 487	0.36	2442 ± 333	827 ± 685	0.25

A3, actual intake during week 3 of cracker snack consumption; A6, actual intake during week 6 of cracker snack consumption; C, calculated intake of cracker consumption; CHO, carbohydrate; D3, absolute displacement of calories/nutrient for week 3; D6, absolute displacement of calories/nutrient for week 6; U, intake of usual diet (baseline diet). Data are means ± SEM. Week 3 data (n=7); week 6 (n=6).

9.2. Under- and Overreporting of Energy Intake

Goldberg Black (2000) equations were applied to energy intake from 7-day food diaries and used physical activity data collected from subjects. **Appendix 8** demonstrates the steps involved in order to identify both individual and group under- and overreporting.

9.2.1 Control group

Of the eight subjects who completed the study, seven subjects returned baseline food diary data and six subjects returned week 6 food diary data. Therefore, Goldberg Black (2000) equations were applied to only six subjects' energy intake data. On an individual basis, one subject was identified as an underreporter at baseline and week 6. Two subjects were identified as underreporters from their week 6 energy intakes.

With regards to the group, the control group was not considered to be underreporters at baseline. However, the calculated mean of the EIrep: BMRest for the group (1.030) was lower than the calculated specific lower cut-off (1.195) which implied overall bias of underreporting in the control group at week 6.

9.2.2 β -glucan group

Of the 13 subjects who completed the study, 11 subjects returned baseline and week 6 food diary data. Goldberg Black (2000) equations were applied to all 11 subjects' energy intake data. On an individual basis, one subject was identified as an underreporter at baseline and week 6. One subject was identified as an over-reporter at baseline. An additional two subjects met the criteria as underreporters at week 6.

With regards to the group, the β -glucan group was not considered to be underreporters at baseline. As with the control group, the β -glucan group were identified as underreporters at week 6. The calculated mean of the EIrep:BMRest for the group (1.109) was lower than the calculated specific lower cut-off (1.246) which implied overall bias.

9.3 Physical activity

Physical activity levels are summarized in **Table 22** for both study groups.

Physical activity levels were significantly greater for the intervention group compared to the control group at baseline, 1109 ± 209 mins, 556 ± 72 mins, respectively ($t(14.6) = -2.5$, $p=0.025$). NB: fractional degrees of freedom is reported because Levene's Test for Equal Variances was not assumed.

Physical activity levels at week 6 of the intervention period did not differ significantly between groups, 870 ± 178 mins and 568 ± 42 mins, ($p=0.122$).

Physical activity levels did not change significantly within the β -glucan and control groups ($p=0.069$, $p=0.885$, respectively).

Table 22. Minutes spent undergoing moderate and intense physical activity for control and β -glucan snack groups at baseline and week 6

	Baseline	Week 6
Control	556 ± 72	568 ± 42
β -glucan	$1109 \pm 209^*$	870 ± 178

Data are means \pm SEM, * $p<0.05$ at baseline, control vs. β -glucan

9.4 Anthropometric Indices

Shapiro-Wilk test of normality determined all anthropometric data as normally distributed, therefore parametric statistical tests were performed (all $p>0.05$).

There were no significant differences between groups for all anthropometric indices at baseline (independent samples t-test, all indices $p>0.05$).

There were no significant differences between groups for all anthropometric indices after 6 weeks of snack consumption (**Table 23**; independent samples t-test, all indices $p>0.05$).

No changes were reported within each group for all anthropometric indices (paired samples t-test, all indices $p>0.05$)

Table 23. Anthropometric indices of control and β -glucan group at baseline and following 6 week snack intervention period

	Baseline		Week 6		p value
	Control	β -glucan	Control	β -glucan	
Weight (kg)	88.6 \pm 4.6	86.6 \pm 5.5	88.8 \pm 4.7	87.1 \pm 5.7	0.83
BMI (kg/m ²)	29.9 \pm 1.3	30.0 \pm 1.2	30.0 \pm 1.3	30.0 \pm 1.3	0.99
WC (cm)	99.7 \pm 3.5	96.3 \pm 3.2	98.3 \pm 2.9	96.3 \pm 3.2	0.67
SAD (cm)	26.4 \pm 0.7	25.9 \pm 1.0	27.3 \pm 0.9	25.9 \pm 1.0	0.38
BF (%)	34.1 \pm 3.4	36.3 \pm 1.4	34.2 \pm 3.3	36.1 \pm 1.4	0.54
Lean (kg)	58.7 \pm 4.9	55.3 \pm 4.0	58.6 \pm 4.6	55.6 \pm 4.0	0.64
Body H ₂ O (%)	46.1 \pm 2.2	44.7 \pm 1.1	46.0 \pm 2.0	44.8 \pm 1.0	0.59

BF body fat, *BMI* body mass index, *SAD* sagittal abdominal diameter, *WC* waist circumference, *H₂O* water. Data are means \pm SEM. Control group n=8, β -glucan group n=13

9.5 Oral Glucose Tolerance Test

All subjects provided full data for OGTT. Shapiro-Wilk test of normality determined the OGTT data as normally distributed, therefore parametric statistical tests were performed ($p>0.05$)

There were no significant differences in fasting blood glucose between the control and intervention group at baseline and post intervention (**Table 24**). There were no significant

changes within groups for fasted blood glucose between baseline and post intervention (control $p=0.51$, β -glucan $p=0.99$, paired samples t-test).

Table 24. Fasted blood glucose (mmol/L) for control and intervention group at baseline and post intervention

	Control	β -glucan	p value
Baseline	5.1 ± 0.2	4.9 ± 0.2	0.47
Post	5.2 ± 0.2	4.9 ± 0.1	0.19

There were no treatment x time effects at baseline between groups (**Figure 21, panel A**). There was no difference in glucose AUC between the control and β -glucan group at baseline, $816 \pm 21 \text{ mmol} \times \text{L}^{-1} \times \text{min}$ and $832 \pm 40 \text{ mmol} \times \text{L}^{-1} \times \text{min}$, respectively, $p=0.72$ (**Figure 21, panel B**).

There were no treatment x time effects at post intervention between groups (**Figure 21, panel C**). There was no difference in glucose AUC between the control and β -glucan group post intervention, $861 \pm 42 \text{ mmol} \times \text{L}^{-1} \times \text{min}$, $800 \pm 29 \text{ mmol} \times \text{L}^{-1} \times \text{min}$, respectively, $p=0.26$ (**Figure 21, panel D**).

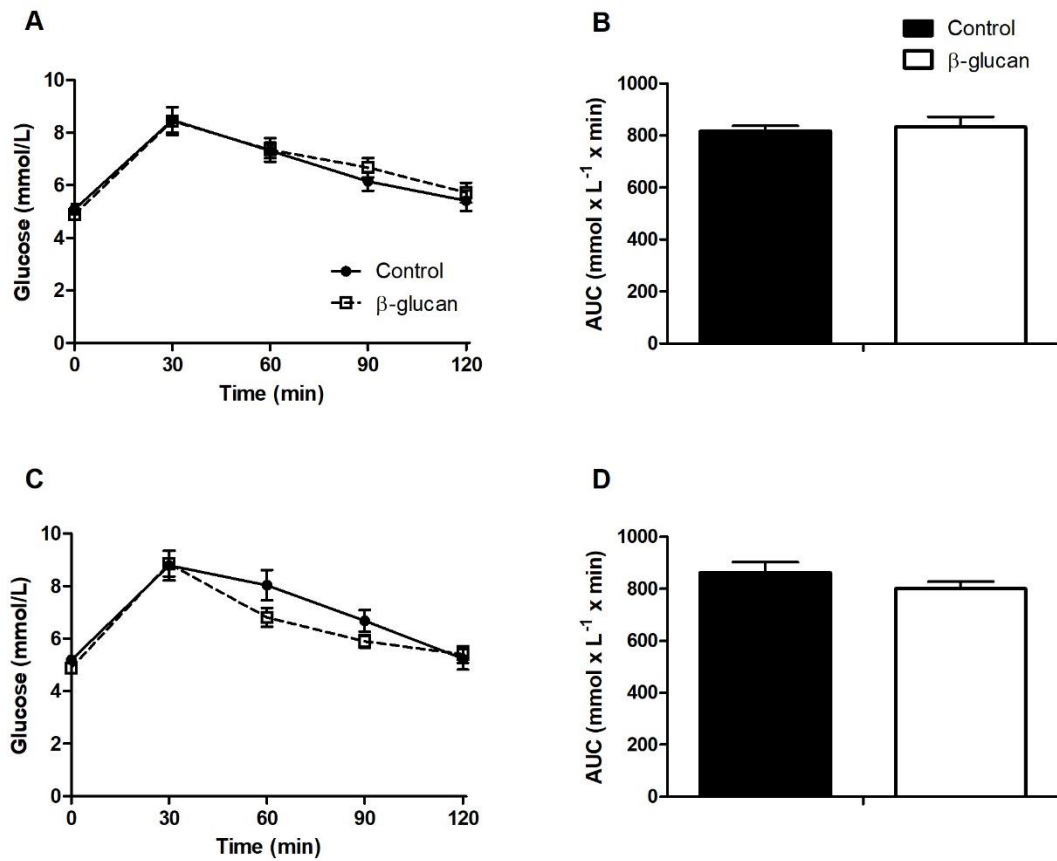


Figure 21. Oral glucose tolerance test at baseline (A) and post intervention (C). Area under the curve for baseline OGTT (B) and post intervention (D). Treatment x time data were analysed with two-factor ANOVA, with treatment and time as factors. Independent samples t-test was performed to compare AUC between groups. Data are means \pm SEM. n=8 control group, n=13 β -glucan group.

9.6 Blood Pressure

Shapiro-Wilk test of normality determined the BP and heart rate data as normally distributed, therefore parametric statistical tests were performed (all $p > 0.05$)

9.6.1 Systolic Blood Pressure

Baseline SBP did not differ between control and β -glucan groups, 123 ± 4 mmHg, 118 ± 4 mmHg, respectively ($p=0.49$).

Following the intervention snack period, SBP did not differ after 6 weeks, 124 ± 3 mmHg, 120 ± 5 mmHg, respectively ($p=0.46$).

9.6.2 Diastolic Blood Pressure

Baseline DBP did not differ between control and β -glucan groups, 79 ± 3 mmHg, 77 ± 4 mmHg, respectively ($p=0.67$). Following the intervention snack period, DBP did not differ after 6 weeks, 78 ± 3 mmHg, respectively 78 ± 4 mmHg ($p=0.93$).

9.6.3 Heart Rate

Baseline heart rate did not differ between control and β -glucan groups, 65 ± 2 bpm, 72 ± 3 bpm, respectively ($p=0.20$).

Following the intervention snack period, heart rate did not differ after 6 weeks, 68 ± 3 bpm, 74 ± 3 bpm ($p=0.21$).

There were no statistical changes in SBP, DBP or heart rate within the control group ($p=0.80$, 0.67 , 0.32) or β -glucan group ($p=0.67$, 0.76 , 0.47 , respectively).

9.7 Subjects' Sensory Evaluation of Snacks

The spider diagram in **Figure 22** presents the end of study evaluation of both snacks consumed by study subjects. Both snacks differed only marginally in terms of overall liking.

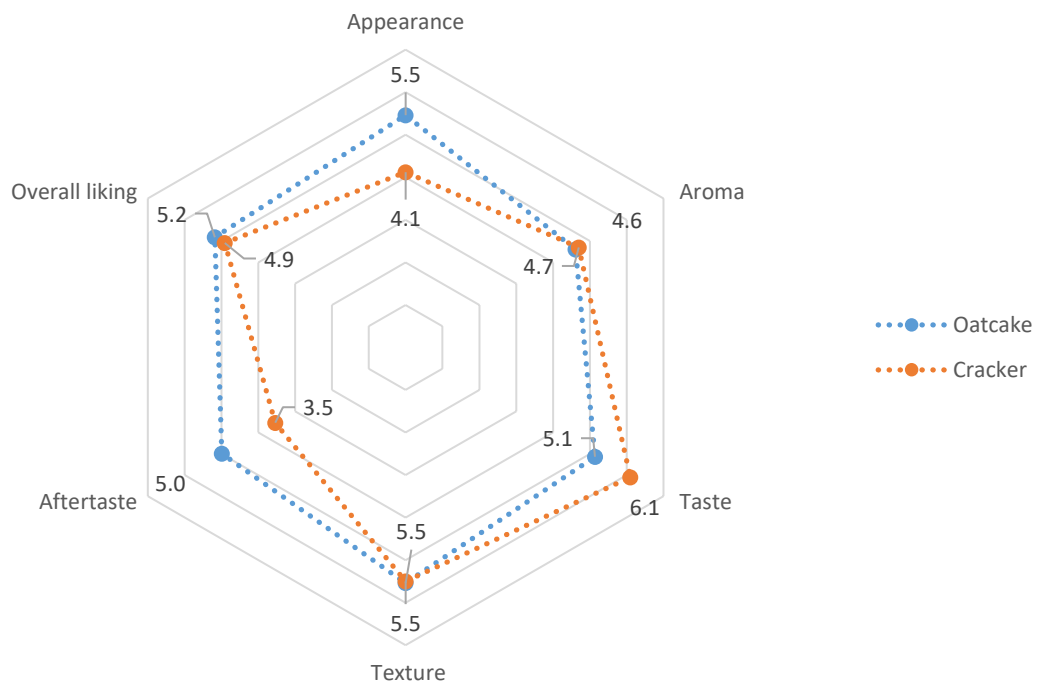


Figure 22. Subject’s evaluation of test snacks. Ratings are scored out of maximum of 7, data are means, n=13 oatcakes, n=8 for cracker

9.7.1 Appearance

The overall appearance of the oatcakes were better received than the control cracker snack. One subject who received the oatcake snacks especially liked the number of oatcakes supplied per pouch as, “*there were enough to snack on throughout the day.*” One subject noted that the control cracker was “easily broken” and for this reason she would often consume the crackers on their own without toppings. Positive comments about the appearance of the oatcake included the shape and overall size/thickness, however two subjects mentioned that they would have preferred a thinner oatcake. Three subjects commented on the ‘paleness’ of the oatcake, and disliked the look of the ‘burnt edges’.

9.7.2 Texture

The texture of both snacks were perceived to be very similar. Subjects who received the crackers found them easy to eat on their own without any toppings, which was in contrast to the oatcakes. The crunchiness of the oatcakes were seen as a positive (n=4). One subject enjoyed “*nice crunchy bits of oats*” in the oatcake snack.

9.7.3 Aroma

The ratings of aroma for both snacks were very similar. Only three comments were received regarding the aroma of the snacks. Two subjects thought that the oatcakes had an ‘oaty’ smell, which was perceived as positive. One subject commented that the oatcakes had a good “*toasted nutty*” smell.

9.7.4 Taste

Ratings of taste were higher for the cracker compared to the oatcake. Subjects generally enjoyed the “*saltiness*” of the cracker, however this was disliked by two subjects. One subject commented that the oatcake “*did not taste any different to other oatcakes*” which can be perceived as positive since the oatcakes contained more fibre than commercially available oatcakes. One subject enjoyed how the oatcakes “*tasted less salty than other oatcakes*”. Most subjects (n=9) said that they preferred to have a topping on the oatcakes. Examples of common toppings were cheese (cheddar, spreading cheese), hummus and butter/margarine spread. Toppings were necessary as most subjects (n=7) found the oatcakes “*dry*” to consume on their own.

9.7.5 Aftertaste

Only three comments were made regarding the aftertaste of the snacks. Two subjects commented that the control snack had a slight ‘oily’ aftertaste. One subject thought that there was a sweet aftertaste following oatcake consumption.

9.7.6 General comments

Generally, subjects enjoyed both snacks in terms of taste and texture but found consuming the snacks every day monotonous. One subject said she would buy the oatcakes but would not consume them in the same quantity every day. Two subjects suggested that the oatcakes could be improved by adding a flavour (cheese and/or black pepper).

Chapter 10

10.0 Effects of a six week-intervention with novel β -glucan-enriched oatcake snacks on daily energy intakes, body composition and markers of metabolic health in overweight and obese individuals: a pilot study (Study B): Discussion

10.1 Energy Intake

The introduction of the high fibre and low fibre snacks did not result in a significant change in mean energy intake between or within groups over 6 weeks. This suggests that subjects compensated for a portion of the calories in the study snacks by reducing their energy intake. The energy intake data from the current study is in alignment with findings of a similar study design. Smith et al. (2008) report that there were no significant differences between energy intakes following ingestion of two concentrated barley β -glucan beverages consumed in adjunct to normal, self-selected diets of overweight mild hypercholesterolaemic subjects. High-MW and low-MW barley β -glucan were consumed by mixing β -glucan concentrate with a beverage and consumed either with a morning or evening meal for six weeks. Although energy intake was quantified by a 24-hour recall method, a recent study by Mosikanon et al. (2017) also report no effect of yeast β -glucan (2 g per day) over a six week supplementation period in overweight and obese subjects. Even when β -glucan was supplemented as part of an energy-restricted diet in overweight females for 12 weeks, neither 5–6 g nor 8–9 g per day of β -glucan incorporated into cereals had any effect on energy intakes (Beck et al. 2010). As discussed in study A, acute changes have been reported in energy intakes following consumption of β -glucan, ranging from 85 kcal to 244 kcal (Beck et al. 2009a; Geliebter et al. 2015; Rebello et al. 2016a). It is possible that small compensations are made following β -glucan consumption over the first few hours subsequent to ingestion but when food records are analysed small energy deficits are perhaps masked by food choices made throughout the remainder of the day. Along with the limitations of using food records to assess dietary intake,

perhaps this is why changes in energy intake are not detected in longer-term β -glucan supplemented studies. Yet despite this, other soluble fibres, such as polydextrose, have reduced energy intake in lean subjects over 14 days (Astbury et al. 2014) and 21 days (Costabile et al. 2012). As with β -glucan, polydextrose is a soluble fibre, however they are not directly comparable fibres as they have different physiochemical properties. For example, the average MW of polydextrose is 200 g mol^{-1} (Aidoo et al. 2016). Therefore, polydextrose and β -glucan may exert different degrees of luminal viscosity after consumption, thus influencing appetite.

For a food that influences short-term satiety to be useful in successful body weight management, studies must be able to show that, under free-living conditions, the exchange of usual for functional foods results in reduced daily energy intake. Furthermore, reductions in daily energy intake must be sustained when the food is consumed on a daily basis. The results from the current study suggest that daily consumption of oat β -glucan does not influence energy intakes of free-living overweight and obese healthy subjects. However, as the sample size of energy intake data in the current study is small, results should be interpreted with caution given that energy intake is an outcome measure of potentially large variance, and as a result of this a much larger sample size would be required to identify small changes in energy.

It is often suggested that the consumption of snacks is an important contributor to weight gain. This suggestion is supported by experimental studies that indeed show that consumption of foods between meals does not lead to adequate adjustments in subsequent energy intake (Whybrow et al. 2007; van Dongen et al. 2010). These findings suggest that snacks are consumed in addition to meals and therefore lead to a higher daily energy intake. Observational studies, however, do not provide consistent evidence on the associations between snack consumption and body weight status (Berteus et al. 2005; Kong et al. 2011). The current study supports the suggestion that the addition of both high and low fibre snacks to habitual diets of overweight/obese individuals does not significantly impact on energy intakes or body

composition indices. Interestingly, oatcakes and crackers are food items usually consumed with toppings, which have the potential to increase the caloric intake of the snacks, however no effect on overall energy intake were reported in the current study. Again, perhaps subjects compensated for a portion of the calories in their diet to accommodate for snack toppings.

Daily consumption of β -glucan-enriched oatcakes incorporated into self-selected diets of overweight and obese free-living subjects did not lead to an improvement in nutrient profiles when compared to the control group. As with reported energy intakes, there was no effect between groups on energy displacement or any other nutrient profiled, thus the β -glucan-enriched snacks did not have a favourable effect on estimated daily energy intakes or nutrient intakes. Interestingly, there were significant displacements identified within the β -glucan snack group for energy. When energy intake during snack consumption was compared to estimated calculated intakes (usual diet plus snacks), there was a displacement of total energy of 500 kcal during week 3 and 487 kcal during week 6. Additionally, there was a considerable displacement in total fat (22-23g) during week 3 and 6 within the β -glucan snack group. Total CHO also demonstrated displacements of 57 g and 53 g during weeks 3 and 6, including a displacement of 17 g sugars. These findings however should be interpreted with caution; firstly, the sample size is small, thus changes in nutrient displacement are based on very few subjects, and secondly, misreporting of energy and nutrient intakes should also be considered. Albeit if these displacements were to be maintained, they may have positive health outcomes.

Despite the β -glucan snack group being significantly more physically active at baseline, there were no differences in time spent undergoing physical activity between groups at week 6, therefore it can be suggested that physical activity did not have an effect on mean group energy intakes. However, it should be considered that unlike energy intake assessment, physical activity was not assessed during week three, thus physical activity at this timepoint is unknown. Snacks containing β -glucan did not alter energy intake in overweight subjects,

neither did the low fibre control, thus, there was no effect of consuming 4.46 g oat β -glucan daily on energy intakes.

10.1.1 Underreporting

With regard to Goldberg-Black analysis performed to identify under- and overreporting (**Chapter 9, section 9.2**), the low basal metabolic rate quota (EI/BMR) in both groups is indicative of a systematic underreporting. Given the small sample size of the subjects who completed the study, it was not appropriate to analyse energy intake data excluding under- and overreporters. If under- and overreporters were excluded from analysis, the energy intake data for the groups receiving the control and intervention snacks would only be three and seven subjects, respectively.

A misreporting of the daily energy intake can lead to uncertainties in the calculated values for specific nutrients, and it can have an impact on the results of total energy intake obtained in the current study. Thus, as mentioned dietary intake data in the current study should be interpreted with caution. Underreporting is in many cases specific, since amounts of fat and sugar rich foods suffer more from underreporting than fruit and vegetables (Poppitt et al. 1998; Heitmann et al. 2000). In addition, previous studies specifically identify underreporting between-meals snacks (Beerman and Dittus 1993; Poppitt et al. 1998). Underreporting may also be due to the measurement in itself imposing behavioural changes (Hadrévi et al. 2017). In a study by Macdairmid and Blundell (1997), it was reported that almost half of the study sample (46%) of subjects admitted to intentionally underreport or alter their food intake in some way as a result of having to record their food intake. Furthermore, in the same study, it was suggested that subjects underreported for two reasons; firstly, subjects felt diet conscious (“feeling too embarrassed”) to record all foods, and secondly, recording food intake was too time consuming and inconvenient. Nowadays, there is a large social pressure for individuals to conform to what society deems as appropriate dietary behaviour (Higgs 2015), and therefore the magnitude of underreporting in nutrition studies is most probably greater at present than

reported by Macdairmid and Blundell 21 years ago. On the other hand, when conducting diet recordings, subjects may reflect on their food choices and food-quantity and deviate from their normal food choices or portion sizes. While this may lead to less reliable results for estimation of normal daily energy intake, it also points at diet recordings as an option to nudge a healthier lifestyle, which would ultimately be of benefit to the subject. However, subjects in the current study did not lose weight, which suggests that behaviour change did not influence physical eating, and additionally, with reference to underreporting, subjects were most likely consuming more to maintain energy balance.

Despite the drawbacks of study recruitment and study power, the test study snacks were matched to provide equal amounts of kcal, CHO, protein and fat with only minor discrepancies between macronutrient and calorie contents, which was a strength of the study. The oatcakes manufactured by Nairn's Oatcakes Ltd. resembled typical oatcakes (**Figure 18, Chapter 8**), therefore subjects were blinded to the β -glucan-enrichment. The control crackers however looked less convincing to subjects, who were aware that the study was supported by Nairn's, therefore it is possible that subjects in the control group of the study may have suspected that their snack was not a new test product of interest. Equally, subjects in the intervention group of the study may have expected to see beneficial effects. Nevertheless, this is the first study to investigate the effect of β -glucan-enriched oatcakes on energy intake in a group of free-living overweight and obese subjects.

10.1.2 Dietary Fat Intakes

There were no significant changes in total dietary fat between groups. Total fat did not increase as much as would be expected by adding β -glucan-enriched oatcake snacks (containing approximately 9 g of fat) to subjects' daily diets. Within the intervention group, total fat intakes remained relatively unchanged throughout the study, with an average daily baseline intake of

86 g and 83 g during the study (contributing to 38% and 36% total energy intake). Within the control group however, there was a reduction of 17 g total fat intake between week three and six of the study period, albeit non-significantly (102 g vs. 85 g). This suggests that perhaps subjects compensated for a portion of the calories in the control crackers by reducing their intake of other fat containing foods. However, the deficit of 17 g fat is an absolute value, and the week 6 intake of 85 g of total fat still contributed to 36% of total energy intake. Nevertheless, reducing the total fat content of the diet by 17 g fat equates to 153 kcal, which if maintained, would be beneficial to the overall diet as DRV for total fat should not exceed 35% TE.

It is recommended that percent of energy consumed from food does not exceed 10% from SFAs (SACN 2018). In the current study, there was a significant difference in total fat intakes (12.1 g) at week three when β -glucan oatcake snacks were consumed compared to the control snack (26.2 ± 2.8 g vs. 38.3 ± 5.5 g, respectively), suggesting that the β -glucan snacks reduced SFA intakes. This is also in alignment with displacements in total fat within the β -glucan group, therefore it can be suggested that displacement in total fat may be attributed to reductions in SFA. However, this was not apparent at week six, as SFA did not differ between groups. The change in SFA intake at week three following the β -glucan snacks was accompanied by significant reductions in TFA between groups. A small but statistical difference of 0.6 g TFA was reported between snack groups at week three. As with SFA, statistical differences in TRA were not apparent during week six, suggesting that the ability of β -glucan snack to modulate SFA and TRA was not sustained consistently over the duration of the intervention period. The control crackers contained more SFA (4.55 g) than the β -glucan-enriched oatcakes (2 g). Therefore, it can be suggested that perhaps reductions in SFA intakes were seen in the β -glucan snack group as the oatcakes (consumed daily) contained less SFA.

The percent total energy from SFA in the current study are in line with (and slightly higher) than population intakes of SFA, with the most recent figures from SACN (2018) reporting

12.1 % total energy from SFA in UK adults aged 19-64 years. According to SACN (2018), one of the main contributors to SFA intake are cereals and cereal products, including biscuits, buns, cakes, pastries, fruit pies and puddings. In order to reduce SFA intakes, it is advocated that individuals reduce consumption of foods high in SFA and replace them for a low SFA containing food alternative. Therefore, the β -glucan-enriched oatcakes could be a healthier alternative snack, providing not only 2 g SFA but almost 9 g of total fibre (of which 4.46 g are β -glucan) and less than 1 g of sugar. Oatcakes may be particularly useful snack for individuals who are overweight, since SFA intakes are associated with CVD risk. Yet, this is currently a controversial topic as robust evidence has emerged highlighting no relationship between increased SFA intake with neither T2DM, ischemic stroke or coronary heart disease among apparently healthy adults (de Souza et al. 2015). However, it should be considered that studies featured in this particular meta-analysis data included prospective cohort studies with subjects who had experienced CV events (i.e., stroke) and therefore do not match the health profiles of the subjects in the current study.

10.1.3 Dietary Fibre Intakes

In 2015, the Scientific Advisory Committee on Nutrition (SACN 2015) increased the UK recommendation for dietary fibre intakes to 30g per day. At baseline, fibre intakes of the subjects in the current study were below the recommendation of 30 g per day, with mean intakes of fibre estimated to be 23 g and 20 g for control and intervention groups, respectively. This could be expected, as studies suggest that low daily fibre intake is associated with overweight and obesity (Alfieri et al. 1995; Howarth et al. 2005; Hadrévi et al. 2017). There were no significant changes in daily average fibre intakes between or within groups over the six week period, with fibre intakes of 26 g during weeks three and six in subjects who consumed the β -glucan-enriched snacks, and 24 g and 21 g during weeks three and six in the control group. An increase in fibre was anticipated in the intervention group as the β -glucan-

enriched oatcakes contained 8.8 g total fibre, which were instructed to be consumed daily. Albeit from a within group perspective, dietary fibre intakes were negatively displaced within the β -glucan snack group. During weeks three and six, it is estimated that 11 g of fibre was displaced during snack consumption. This suggests that within the intervention group, subjects may have reduced consumption of other fibre-containing foods whilst consuming β -glucan snacks. However, displacement data showed there were no statistical differences between groups, therefore the displacement of fibre within the intervention group cannot be attributed to the β -glucan oatcake alone.

It should be considered that fibre intakes were estimated from food diaries and, as mentioned, group underreporting was present in the current study. Therefore, fibre intakes are likely to be slightly lower than reported. Nevertheless, dietary fibre intake in the present study were based on 7-day food diaries, unlike previous studies that have used as little as three days to record food intakes to estimate fibre intake (Alfieri et al. 1995; Yang et al. 2010). Dietary records are the most common method used in literature for assessing fibre intake. Day and colleagues (2001) validated 7-day diet diaries and a FFQ against urinary biomarkers of nitrogen, potassium, and sodium, and found the records to be more accurate than the FFQ. However, as with energy intakes, it should be acknowledged that using weighed food intake may have provided more accurate measures of foods and drinks consumed during study periods.

10.1.4 Further investigations of β -glucan on energy intake

In the current study, subjects were asked to consume their test snack throughout the day, at a time of their choosing. From a mechanistic perspective, perhaps a better alternative would have been to ask subjects to consume their test snacks 30-90 minutes before a main meal, i.e., lunch or dinner. Since there were no effects on energy intakes reported, this alternative

suggestion may be more effective in reducing food intake following ingestion of β -glucan-enriched snacks as the β -glucan would have had time to alter the viscosity of stomach contents.

Additionally, perhaps the current study could have explored the short-term appetite effects of the β -glucan-enriched oatcakes in an acute satiety study setting throughout the six week intervention period. This would have allowed identification of whether an acute eating-inhibitory effect was present following consumption of β -glucan-enriched oatcakes. A strength of conducting such an experiment would be avoiding the drawbacks of using food diaries to record energy intake. It is well known that overweight subjects tend to underreport their intake by means of diaries (Goris et al. 2000; Poslusna et al. 2009) therefore offering an *ad libitum* meal would be a better option, particularly since in the current study both groups were classified as underreporters at both individual and group level.

A limitation of the present study was that subjects had only one choice of snacks for six weeks, and during the intervention liking of the snacks may have decreased. There is a risk that the subjects could have eaten other snacks to compensate for the boredom owing to the same flavour and texture of the oatcakes and/or crackers, and consequently did not lose weight. Although there were increases in flatulence and bloating, as well as effects on stool consistency during weeks three and six of the study, this indicates that fermentation in the colon was occurring in the β -glucan snack group. Future studies should include plasma and stool samples to measure levels of satiety hormones, SCFA or changes in microbiota composition to speculate about possible biological mechanisms. This is particularly pertinent since it is suggested that long-term ingestion of soluble fibre may lead to increased satiety due to increased proliferation of GLP-1 and PYY producing L-cells (Kaji et al. 2011; Kuwahara 2014). Kuwahara (2014) suggests that this can only occur after long-term ingestion of soluble fibre (>4 weeks), as fermentation can take a number of days to occur and only then can this affect GLP-1 and PYY production. Therefore, continuous intake of fermentable fibre in the

diet is considered important for the expression of GLP1-and PYY-secreting L-cells in long-term energy homeostasis.

10.2 Adverse effects

Dietary fibre may also have potential adverse dietary effects, such as reduced absorption of vitamins, minerals, proteins and energy as well as GI side effects (Lattimer and Haub 2010). This applies especially when dietary fibre is consumed in excess and within an inadequate period of time to allow the GI tract to adapt. However, it is unlikely that healthy, adult individuals who consume dietary fibre within the recommended range would have difficulties with nutrient absorption (Slavin 2008).

Since β -glucan is fermented by bacteria in the colon, its consumption can cause flatulence, bloating, and abdominal cramps, especially when β -glucan intake has suddenly increased. In several human intervention studies, the influence of β -glucan on GI symptoms was measured. No GI side effects were noted by overweight women after 4 weeks of intake of an oat β -glucan-enriched diet, 2.31 g per day (Robitaille et al. 2005). Daily ingestion of 3 g of oat β -glucan by patients with T2DM for 3 weeks did not produce any adverse effects, including GI symptoms (Liatis et al. 2009). In the current study, the minimum daily intake of β -glucan was 4.46 g, however more β -glucan may have been consumed from other foods, such as oats. Six subjects reported GI effects, including flatulence, bloating and changes in stools. One subject rated their bloating as severe but decided to continue to participate in the study. The consumption of higher doses of oat β -glucan (7.7 g per day) for 12 weeks by subjects did not increase the incidence of cramping and bloating in a study by Maki et al. (2007). However, the incidence of diarrhoea and loose stools was significantly higher in the β -glucan group than in the control group. On the other hand, Keogh and colleagues (2003) report no adverse effects following 10 g β -glucan per day. These studies suggest that consumption of a daily dose of up to 10 g of β -glucan is well tolerated. Higher doses of β -glucan might not be harmful to health, but doses

higher than 10 g per day are usually not ingested in a single meal (Cloetens et al. 2012). Food compliance, mentioned in almost all of the intervention studies discussed here, was reported as good or very good, supporting the idea that β -glucans are well tolerated. One study observed a significantly lower compliance in the β -glucan group compared with the control group (Maki et al. 2007); however, the authors suggested that the lower compliance as well as the greater number of dropouts in the β -glucan group was due to the low palatability of the β -glucan diet rather than to low tolerance.

10.3 Body weight and body composition

As the effect of β -glucan on satiety is still unclear, its effect on body weight regulation is less clearer. In the current study, there were no significant changes in body composition assessment indices following daily consumption of oatcakes containing 4.46 g oat β -glucan for six weeks.

Intervention studies that aimed to achieve an isocaloric diet did not find significant effects of β -glucan, administered at a daily dose ranging between 2.3 g and 10 g, on body weight, BMI, or WC (Maki et al. 2007; Cloetens et al. 2012). For example, anthropometric indices remained unchanged in 26 patients with elevated BP (mean baseline BMI 32.6 ± 1.0 kg/m²) during a 12-week intervention period with 7.7 g per day oat β -glucan (Maki et al. 2007). In a study that used the same duration of β -glucan supplementation as the current study, the body weight of hypercholesterolaemic subjects also remained constant after a daily intake of 6 g of oat β -glucan (Queenan et al. 2007). In a study on diabetic patients, the supplementation of β -glucan from oats, at a dose of 9 g per day over a longer period of time (24 weeks), did not have any significant effect on body weight (Ripsin et al. 1992). In another study on hyperlipidaemic patients, weight differences were not observed following the consumption of a diet rich in oat β -glucan (8 g per day), over 1 month only, as compared to the control group (Jenkins et al. 2002). It should be noted that the body weight was not the primary concern of these studies as they focused on changes in blood sugar or blood lipids.

Conversely, Smith et al. (2008) reported a statistical decrease in body weight following six weeks consumption of 6 g barley β -glucan daily as an adjunct to normal, self-selected diets of free-living overweight subjects. Subjects who consumed the high MW barley β -glucan concentrate lost on average 0.41 kg, compared to subjects who consumed low MW barley β -glucan concentrate. The group who consumed LWM β -glucan increased their weight by 0.37 kg when compared to baseline body weights. Interestingly, changes in energy intake did not parallel weight gained or lost in this study. No significant decreases in energy intakes were reported in the group who consumed high MW, even though they lost 0.41 kg over the six week intervention period. Conversely, within group analysis identified that the group who gained weight (low MW) significantly reduced their energy intakes by approximately 173 kcals. Foods containing dietary fibre tend to provide bulk to the diet (Dhingra et al. 2012), without providing as many calories, fat and added sugar. The combination of bulky diet, the increased chewing associated with these highly-dense foods and their gel-forming action all lead to stomach distension which may signal an individual to stop eating and thus, decrease overall energy intake. Perhaps a small but significant decrease in body weight occurred in the study by Smith et al. (2008) because subjects benefitted from the higher viscosity of the high MW β -glucan concentrate, yet no significant reductions in energy intake were reported. A strength of the current study is that energy intake was measured on three occasions, each with a 7-day food diary at baseline and during week three and six (final week) of the intervention period. Therefore, unlike the study by Smith et al. (2008), more conclusions can be drawn with regards to energy intakes throughout the intervention period, with food intake data collected throughout the study.

Liatis et al. (2009) also report a significant decrease in mean body weight (-1.03 kg), BMI (-0.38 kg/m²), and WC (-1.63 cm) in 23 T2DM patients (BMI of 29.6 ± 4.8 kg/m²) after a daily intake of only 3 g of oat β -glucan for 3 weeks compared with baseline but not with the control group. However, as the test breads used in the study by Liatis et al. (2009) were not matched

appropriately for CHO and total calories, there is a possibility that the intervention group lost weight and reductions were seen in BMI and WC because test breads contained fewer calories and CHO content. The β -glucan bread contained 25 g CHO and 170 kcal, whilst the control bread had almost double CHO content (48 g) and 245 kcal. According to Liatis et al. (2009), subjects in the control group were instructed to slightly reduce their CHO intake from other dietary sources to compensate for the discrepancy in test bread CHO content. Yet, a major limitation of this study was that dietary intakes or exercise were not monitored during the study. Therefore, it is unknown whether the control group actively tried to reduce their CHO intakes. Additionally, due to the lack of diet and exercise data, it is not possible to recognise β -glucan as the cause of weight loss, or BMI and WC reductions. The lack of dietary assessment is common in most studies that have investigated the effect of β -glucan consumption in the medium term (Smith et al. 2008).

Findings from a study by Robitaille et al. (2005) are in agreement with the current study. Following a four week supplementation period in overweight subjects (2.31 g per day β -glucan incorporated into muffins) no changes were reported in body weight, BMI or WC. No effect on anthropometric indices were reported, which were accompanied by no significant changes in energy intakes or physical activity. However, exact data on physical activity of the subjects was not presented in the findings of Robitaille et al. (2005), therefore it is not possible to comment on how physically active both groups were. This is pertinent since the authors report a significant decrease in body weight and BMI of subjects within the control group (baseline vs. four weeks, -0.66 kg and -0.25 kg/m², respectively).

In the current study, dietary intakes were assessed on three occasions, with body composition measured twice, pre and post study. Body composition was assessed by measurement of weight, BMI, body fat percentage and body water content. Measurement of body fat is important in assessing the effectiveness of dietary interventions, as body weight alone (and BMI) do not distinguish between fat-free mass and fat mass, therefore it is not feasible to attain

whether fat-free mass and/or fat mass has been lost. This is particularly important as fat mass and its distribution poses greater risk of CV and metabolic disease (Goossens 2017). Accumulation of adipose tissue in the upper body (abdominal region) is associated with the development of obesity-related comorbidities and even all-cause mortality (Zhang et al. 2008). In contrast, population studies have shown that accumulation of fat in the lower body (gluteofemoral region) is associated with a protective lipid and glucose profile as well as a decrease in CV and metabolic disease prevalence after adjustment for total body fat mass (Snijder et al. 2004; Yusuf et al. 2005). One study has investigated the effect of a 12 week intervention of consuming a mixture of rice and pearl barley with a high β -glucan content (7 g per day) on body composition indices. The hypercholesterolaemic Japanese men, with baseline LDL cholesterol levels of > 4.1 mmol/L, who took part in the study experienced a significant reduction in BMI (-0.3), WC (-1.2 cm), and visceral fat (-10.6 cm²) in comparison to the placebo group who consumed rice alone (Shimizu et al. 2008). The mean of calorie intakes or walk counts (daily steps) in the test group were not significantly different from those in the placebo group, thus changes in BMI, WC and visceral fat were perhaps attributable to the barley content of the diet. It would be assumed that an increase in viscosity of the meal containing β -glucan would induce satiation, and therefore a reduction in energy intake. However, in the aforementioned study, no changes were seen in energy intake yet changes in visceral fat were reported, along with reduction in BMI and WC. Perhaps energy misreporting was prevalent in this study, as was not assessed, which may explain the reductions in visceral fat, WC and weight without changes in energy intake and physical activity.

To the researcher's knowledge, the current study is the first study to include SAD as a measure of visceral obesity following β -glucan. Along with WC, there was no effect of β -glucan snack consumption on SAD, which can be explained by no significant decreases in body weight, body fat and energy intake outcomes. Since SAD is an inexpensive and relevant marker of assessing abdominal fat and CV risk (de Souza and Oliveira 2013), gaining insight into the

effect of β -glucan on SAD is particularly pertinent, given that the effect of β -glucan on this marker is unknown. It is acknowledged that gold standard methods i.e., DEXA would be the most appropriate method to assess changes in body composition in overweight and obese adults, however accessing such specialised equipment was not possible in the current study and reflects a limitation.

From the literature discussed, and considering the findings of the current study, it is clear that variations in the food sources of β -glucan, rather than in the dose and the duration of administration, may explain such contradictions in findings and appear as critical determinants of body weight regulation. Small, but significant, reductions in WC and/or body weight have been reported following the consumption of β -glucan-enriched bread, β -glucan concentrate-containing beverages and barley rice, over three, six and 12 weeks, respectively (Smith et al. 2008; Shimizu et al. 2008; Liatis et al. 2009). On the other hand, no effects are reported after consumption of β -glucan in food formats such as muffins, or oatmeal and oat bran, over 3 or 12 weeks (Robitaille et al. 2005; Maki et al. 2007). As with the acute satiating effects of β -glucan, it is likely that the physiochemical properties of the intervention food matrix influences the viscosity of the food bolus containing β -glucan to move transit throughout the GI tract. Thus, since physiologic effects of β -glucans may be altered by food processing techniques, it is important to develop a further understanding of such interactions.

Perhaps dietary intervention studies involving β -glucan should focus on using more sophisticated body composition analysis tools to assess body composition, so that assessment of changes in body fat distribution can be identified. Nevertheless, the current study did measure total body fat percentage and abdominal obesity via WC, which is more informative than studies that measure body weight (and BMI) alone.

At moderate (5-6 g per day) and high (8-9 g per day) doses, the addition of oat β -glucan to an energy-restricted diet did not enhance the effect of energy restriction on weight loss in overweight women after a period of 3 months (Beck et al. 2010). To the researcher's

knowledge, the study by Beck and colleagues (2010) is the only study identified that has investigated the effect of β -glucan incorporated in conjunction with a hypocaloric diet. Therefore, more evidence is needed to explore the efficacy of including β -glucan in weight reducing diets.

The effect of dietary β -glucan on anthropometric measurements remains unclear. Dose, physical properties, i.e., MW, and processing of β -glucans and food matrix may also influence the putative induced changes in anthropometric values. Moreover, the observed changes in anthropometric measurements largely depend on the total energy intake and physical activity and the intake of other components. If body weight loss is observed after a β -glucan intervention period, for the reasons discussed it is difficult to state that β -glucan is the main factor responsible for body weight reduction. Much of the research with β -glucan as a functional ingredient focusses on improvements in parameters such as glycaemic control and hypercholesterolaemia. The research here used 'healthy' overweight and obese individuals, thus greater effects may have been seen in a population with pre-MetS or diagnosed MetS. Subjects presenting with at least two components of MetS may provide more insight into the mechanistic effects of disease establishment. For example, evidence shows that people with pre-diabetes tend to develop T2DM within 10 years and are at increased risk for CVD and mortality even before the development of diabetes. Lifestyle interventions (i.e., dietary manipulations) can delay and halt progression to T2DM, with reported normalization of impaired glucose tolerance as a result of lifestyle modification (Eriksson and Lindgärde 1991; Pan et al. 1997).

10.4 Blood pressure

Consumption of 4.46 g per day of oat β -glucan had no effect on SBP, DBP or heart rates of overweight and obese subjects. As this study focussed on subjects who were overweight and obese it was anticipated that BP may have been in the high-normal category (130 to 139 / 85

to 89 mmHg). In fact, BP taken at baseline and after six weeks of snacking was within the normal BP range for both groups of overweight and obese subjects. The current findings are in line with data from Maki et al. (2007), who report no BP lowering effect of consuming 7.7 g β -glucan. Despite the intervention period being twice as long (12 weeks) and a higher supplemented dose of β -glucan, Maki and colleagues report no significant change on BP in overweight individuals. However, it should be noted that after subgroup analysis ($\text{BMI} > 31.5 \text{ kg/m}^2$), Maki et al. (2007) report reduction in both SBP and DBP between the β -glucan and control treatments. Subjects with $\text{BMI} > 31.5 \text{ kg/m}^2$ in the β -glucan group showed mean baseline to end-of-treatment reduction in SBP and DBP of 5.6 and 2.1 mmHg, respectively. High BMI control subjects showed increases of 2.7 and 1.9 mm Hg. Had the samples sizes of the current study been larger, subgroup analysis could have been performed to look at the impact of $\text{BMI} > 30 \text{ kg/m}^2$ on BP. Nevertheless, the current study did monitor weight status and assessed body composition at the beginning and end of the intervention period, which is not only a limitation of the study by Maki et al. (2007) but also a drawback to the well powered study by Saltzman et al. (2001). Conversely, Smith and colleagues (2008) report a reduction in weight by 0.41 kg in overweight subjects who consumed 6 g β -glucan daily with no changes in BP reported.

It is important that body weight and body composition is monitored in studies that assess BP, as weight loss is a modifiable factor and the first line of defence against high BP. The average individual effect size of BP noted in dietary intervention trials is generally relatively small. For example, Neter et al. (2003) suggest that for every 1 kg weight loss, SBP and DBP would decrease by 1 mmHg. However, these small effects can translate into important reductions in the incidence of hypertension at a population level (Klag et al. 1990). It is estimated that each 2 mmHg reduction in SBP and 1 mmHg reduction in DBP is associated with a 10% reduction in the risk of CVD (BHF 2012). There were no changes in weight in the current study, therefore it is likely that this explains why no effect on BP was reported. However, a study by He et al.

(2004) reports a reduction in BP independent of weight-loss, which suggests there may be a potential mechanism whereby β -glucan directly attenuates BP. This is also supported by a novel study by Mosikanon et al. (2017) following six weeks supplementation of yeast β -glucan.

Low-grade inflammation is a key player on the pathophysiology of CVD. In this regard, it is established that increased production of proinflammatory cytokines, such as interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF- α), modulates vascular function by increasing contractile pathways and also, by decreasing endothelium-dependent vascular relaxation through distinct mechanisms (Schiffrin 2010). Interleukin-10 (IL-10) is a multi-functional cytokine with potent anti-inflammatory properties. In the recent study by Mosikanon et al. (2017), six weeks of supplementation of yeast β -glucan increased IL-10 and reduced IL-6 and TNF- α alongside significant decreases in BP between the control and yeast β -glucan supplemented group. After six weeks, SBP and DBP were significantly lower in the β -glucan group compared to the control group, 129/80 mmHg and 142/86 mmHg, respectively. Since no body weight changes were reported in this study, it can be suggested that perhaps yeast β -glucan altered both pro-inflammatory and anti-inflammatory responses, resulting in BP attenuation. Although more research is required, it is possible that the BP attenuating effects of yeast β -glucan may influence cytokine concentrations that facilitate alterations in BP. Still, however, no direct mechanism has been identified whereby oat and barley β -glucan influence BP.

Dietary changes other than soluble fibre intake could, however, explain some of the BP-lowering effects documented in the literature. For example, reductions in sodium and calcium intake and an increase in potassium intake were observed in overweight subjects who consumed β -glucan daily over 12 weeks (Maki et al. 2007). There were no significant changes in micronutrients in the current study, therefore it can be ruled out that increases in sodium and calcium may have counteracted a potential attenuation of BP. Salt intakes (converted from

sodium) were particularly high in the control group at baseline, with baseline intakes of 8.17 g salt. Intakes during the intervention period (non-significantly) reduced to around 6 g. Salt intakes in the β -glucan group were below the DRV for salt (<6 g) per day. Again, it should be considered that micronutrient intakes were assessed by food diaries, and as mentioned, both groups in the current study contained underreporters. Therefore, there is a possibility that they may have underreported on foods that contained particularly high sodium contents, i.e., convenience foods.

It can be argued that since subjects had normal BP, there would be no clinical benefit of lowering BP that was not high-normal or hypertensive. With this in mind, perhaps focus should be on investigating the effect of β -glucan consumption in individuals who have elevated BP but do not take antihypertensive medications, i.e., are pre-hypertensive.

10.5 Glucose response

The present study shows that the long-term consumption of β -glucan-enriched oatcakes daily had no detectable effect on the glycaemic profile of free-living overweight and obese subjects. The incorporation of 4.46 g of β -glucan in oatcakes, ingested for six weeks, did not alter fasted glucose levels or glycaemic responses over a 120 minute period. Although health claims associated with oats had been approved by the US Food and Drug Administration (FDA) since 1997, the health-promoting effect of oat products is suggested to be achieved only when oat products are consumed regularly for some time and reach a certain oat β -glucan dose threshold in the diet (Sadiq et al. 2008; Cloetens et al. 2012). Yet despite this fact, very few studies have investigated the effects of longer term consumption of oat β -glucan on fasted glucose and glycaemic responses in overweight and obese subjects, with most studies focussing on acute glycaemic responses immediately after β -glucan ingestion. The results of the current study are novel in that they show that consumption of a moderate dose of β -glucan for six weeks had no beneficial effect on fasted blood glucose levels in overweight and obese subjects. No metabolic

effect was seen, which is particularly surprising as daily consumption of 3 g β -glucan has been shown to improve markers of metabolic health, i.e., HDL cholesterol and TGs (Tiwari and Cummins 2011; Whitehead et al. 2014).

In the present study, there was no evidence of an improvement in glucose control, which may be unsurprising because no subjects were diabetic or had impaired glucose tolerance. Weight loss has been shown to positively influence glycaemic control in a number of studies (Rock et al. 2014; McAdam-Marx et al. 2014; Avery et al. 2017). It can therefore be suggested that lack of weight loss in the current study may have deterred any beneficial effect on glycaemic control. Although all subjects had fasted blood samples within the normal range (≤ 5.6 mmol/L), the researcher could not be certain that subjects followed pre-testing protocols, i.e., fasted for 10 hours, avoided alcohol the evening before, or did not participate in exercise prior to testing; all factors that can influence blood glucose concentrations. Moreover, fasted plasma insulin was not measured in the current study, neither were other CVD markers such as TGs, LDL- and HDL cholesterol. Assessment of such CVD markers would be of interest given that postprandial glycaemia and related insulinaemia and lipidaemia are implicated in the aetiology of chronic metabolic diseases such as T2DM and CVD (Blaak et al. 2012).

There is evidence to suggest that oat β -glucan consumption is effective in attenuating blood glucose in subjects with T2DM. Meta-analysis findings by Shen et al. (2016) report that consumption of between 2.5 and 3.5 g oat β -glucan for three to eight weeks improves glycaemic control in individuals with T2DM (Kabir et al. 2002; Liatis et al. 2009; Ma et al. 2013). A reduction of 0.52 mmol/L in fasting blood glucose and 0.21% of HbA1c after 3 to 8 weeks of intervention is reported by Shen and colleagues (2016). Although rather small in size, this small reduction in fasted blood glucose could be relevant for well-controlled T2DM patients. However, the clinical significance of these results remains to be confirmed in the long term, when reduced patient compliance or physiological adaptive changes induced by chronic oat addition may occur. Francelino et al. (2014) highlight that the consumption of greater

doses of β -glucans or smaller doses of β -glucans for longer periods of time may produce better results. For example, an acute dose of 6 g and 8.4 g β -glucan reduced both glycaemia and insulinaemia in T2DM subjects (Tappy et al. 1996). Following a 12 week consumption of 3 g per day β -glucan reduced glycaemia by 46% (Pick et al. 1996), whilst the same dose ingested for four weeks (Kabir et al. 2002) or 3.5 g per day for eight weeks (Cugnet-Anceau et al. 2010) were not effective.

In a study by Battilana et al. (2001), findings indicate that ingestion of diets (for three days) containing 8.9 g per day β -glucan produced only very modest reductions in plasma glucose and insulin concentrations compared to a diet without β -glucan of similar macronutrient composition with equivalent amounts of insoluble fibres when the diets were administered as small meals every hour. Administration of frequent meals with or without β -glucan resulted in similar CHO and lipid metabolism, indicated by no differences in rates of *de novo* lipogenesis or exogenous CHO. This suggests that the lowered postprandial glucose concentrations which are often observed after ingestion of a single meal containing β -glucan are essentially due to a delayed and somewhat reduced CHO absorption from the gut, and do not result from the effects of fermentation products in the colon. In relation to the current study, the acute effects of snack oatcake consumption were not investigated, therefore, as previously mentioned, the acute effects of the β -glucan-enriched test snack warrant further research, particularly since one portion (54 g) provides 27 g available CHO per 4.46 g oat β -glucan. As discussed (**Chapter 6, section 6.2**) 4 g β -glucan per 30 g available CHO can also be taken from the perspective of 0.133 g oat β -glucan is required per gram of available CHO to obtain a reliable effect on glycaemic response. The β -glucan-enriched oatcakes would provide 0.162 g oat β -glucan per gram of available CHO.

10.6 Physical activity

In theory, physical activity should provide an effective method of weight control since it has the potential to induce a negative energy balance by increasing daily energy expenditure (EE). However, it is possible that due to this energy deficit, exercise increases the drive to eat, and a compensation in energy intake occurs in order to match the exercise-induced EE (Blundell et al. 2003). In the current study, there were no changes in physical activity levels within or between groups during the intervention period. It can therefore be suggested that physical activity had no effect on daily energy intakes. It is difficult to compare physical activity levels of subjects in the current study with similar studies, as studies have either not measured the physical activity levels of subjects (Liatis et al. 2009; Ma et al. 2013), or do not report physical activity data in their results (Kabir et al. 2002; Robitaille et al. 2005; Beck et al. 2010). In one study by Shimizu and colleagues (2008), pedometers were worn in order to assess the physical activity of subjects who consumed either high β -glucan barley or control rice for 12 weeks. Average step counts were approximately 7100 and 7700 steps daily for each group, respectively. Although the average BMI of subjects in the study by Shimizu et al. (2008) was lower than subjects in the present study, 25 kg/m², the overweight subjects were walking >7000 steps per day, which equates to around 5 km or more in walking distance per day (1 metre = 1.3 step conversion), thus subjects were not entirely sedentary. Guidelines for physical activity advocate that at least 150 minutes of moderate aerobic activity, such as cycling or brisk walking every week along with strength exercises on two or more days a week that work all the major muscles (legs, hips, back, abdomen, chest, shoulders and arms) should be followed (Bull et al. 2010; NHS 2018). In the current study, subjects reported to engage in between 556 – 1109 minutes of moderate/vigorous physical activities, suggesting that the overweight and obese subjects meet physical activity guidelines. However, a pitfall of the SPAQ used in the current study to assess physical activity is the inability to distinguish between CV-based and resistance-based activities, and thus a breakdown of aerobic and

strength exercises was not possible. Nevertheless, physical activity was measured in the current study using a validated questionnaire (Lowther et al. 1999). It should be noted that there exists more robust means of monitoring physical activities in free-living subjects. The SPAQ, completed retrospectively, quantified total time spent undergoing activities of moderate and/or vigorous intensity only. Light intensity activities, such as casual walking, were not included; only walking of a *brisk* nature was. Using activity monitors (eg., activPAL™) that measure 24/7 EE would distinguish between sedentarism from minimal daily activities, and reduce bias associated with self-reported physical activity levels, thus improving the accuracy of data. Monitoring subject study compliance would be achievable, especially with monitors worn the day prior to test days when strenuous exercise was prohibited.

Many studies have examined appetite, appetite hormone and energy intake responses to acute bouts of exercise, and the general consensus is that in the short-term (24-48 hours) there is not a strong relationship between EE and energy intake (Blundell et al. 2003; Donnelly et al. 2014; Beaulieu et al. 2016). However, attaining more precise data on subject physical activity during dietary intervention studies is important as there is evidence that physical activity may influence appetite by modulating the hedonic response to foods. For example, individuals would report greater pleasure from food consumption following a period of exercise. This has been demonstrated in female dieters who showed increased ratings of pleasantness for a range of foods following exercise (Lluch et al. 1998). Reported perceptions of the tastiness and pleasantness of high CHO (low fat) foods were reported. This adjustment in the appreciation of foods can be considered consistent with a physiological need for CHO which is generated by the utilisation of glycogen during exercise. It should be recognised however that there is still a large gap in the literature with regards to the window of time in which energy compensation may occur.

10.7 Sensory feedback of product

Incorporating significant amounts of fibre into food products constitutes a technological challenge due to the potential deleterious effects on textural quality. According to literature, the main problems associated with adding β -glucan to foods are modifications of texture, sensory characteristics, along with shelf-life (due to water-binding capacity), fat mimetic, anti-sticking and thickening effects. Thickening of the oatcake mixture was particularly problematic in the current study, which resulted in the β -glucan-enriched oatcakes being handmade instead of mass produced. Despite this being a time-consuming process, the end product was (from an appearance perspective) visually and texturally very similar to standard rough oatcakes manufactured by Nairn's. When subjects were asked to rate the sensory characteristics of the β -glucan-enriched oatcakes, taste, appearance, texture and overall liking were well received by subjects, with scores of at least five out of a possible seven. Compared to the control snack, the β -glucan-enriched oatcakes scored better for all sensory attributes except for aroma (marginal difference in ratings of 0.1) and taste. Taste perceptions of test control crackers was rated 6.1, which was higher than β -glucan-enriched oatcakes, which scored 5.1. The higher taste rating for the control snack was most likely due to the saltiness of the cracker. Only two subjects from the control group said they disliked the saltiness of their snack. Evidence suggests that salt enhances the positive sensory attributes of foods, even in some otherwise unpalatable foods as salt makes foods 'taste better' (Henney et al. 2010). However, despite the control cracker snack scoring better on taste ratings, this did not affect overall liking of test snacks as the β -glucan-enriched oatcakes scored higher.

The incorporation of oats into baked products, such as bread, baked goods, and dough, has been widely tested for their effect on blood lipids, glucose and appetite (Havrlentová et al. 2011). The integration of β -glucans in baked products is encouraging, ameliorating both sensory characteristics and health properties of products at a maximum concentration of 20%. For example, when oat flour has been substituted for 10% fine wheat flour in bread, product

quality improved in terms of crust colour, bread softness and taste in a study by Gormley and Morrissey (1999). A positive effect of oat β -glucan on the sensory characteristics of biscuits has also been described (Lee et al. 2009). Although the test oatcakes in the current study were not compared to a standard rough oatcake manufactured by the same company, which is a weakness of the study, the sensory characteristics of the β -glucan-enriched oatcakes were positive. This is particularly beneficial as evidence of the effects of β -glucan in milk and milk products on sensorial properties have been reported with variable results (Önning et al. 1999; Mårtensson et al. 2001; Biörklund et al. 2005; Mårtensson et al. 2005). Oat milk containing β -glucan (0.5 g/100 g) was well perceived and received similar sensory evaluation as the control drink (<0.02 g β -glucan/100 g) in a study by Önning et al. (1999). Sensory evaluations were higher for the milk beverage (500 mL) enriched with 5 g β -glucan when compared to the drink enriched with 10 g of oat and barley β -glucan (Biörklund et al. 2005). However, milk enriched with 5 g β -glucan had similar sensorial characteristics to the control drink. Evidence provided from study A in this thesis suggests that oat β -glucan was not well received when incorporated into milk and Greek-style yoghurt, with significantly lower ratings of palatability, taste and visual appeal. As discussed in **Chapter 6 (section 6.1.3)**, sensory cues based on a food's sight, smell, taste and texture are operational before, during and after an eating event (McCrickerd and Forde 2015). With this in mind, it can be suggested that the β -glucan-enriched oatcakes could be a more effective means of delivering more fibre (and β -glucan) to the diets of individuals, as this solid baked food matrix was better perceived in terms of sensory characteristics than compared to the food matrix from study A. With regard to feasibility, a product needs to present itself as palatable to be consumed repeatedly, and unfortunately this was not the case for the food matrix tested in study A.

Chapter 11

11.0 Summary and Concluding Remarks

The aim of this PhD thesis was to investigate the role of oat β -glucan on energy intakes in both the acute and medium-term (six weeks). Two breakfasts varying in oat β -glucan content and viscosity were selected to examine the acute effects of soluble fibre β -glucan on *ad libitum* eating, subjective appetite, in addition to post-prandial glucose and plasma insulin and GLP-1 responses (study A). A novel β -glucan-enriched oatcake was manufactured and provided for consumption over a six week period to investigate the impact of daily consumption of β -glucan on energy intakes and physiological markers associated with MetS (study B).

In study A, β -glucan enrichment of a semi-solid breakfast consisting of Greek-style yoghurt and Rice Krispies with milk, had no effect on subsequent eating; consuming 4 g oat β -glucan at breakfast had no effect on *ad libitum* eating, or throughout the remainder of the day in healthy-normal weight subjects. β -glucan enrichment did however significantly increase subjective appetite ratings of satiety and fullness, with significant decreases in both glucose and insulin over a 90 minute post-prandial period. GLP-1 was significantly decreased at 90 minutes following β -glucan-enriched breakfast. The increased viscosity of the test breakfast may not favour the release of GLP-1 from enteroendocrine cells, thus this may explain why GLP-1 was attenuated following β -glucan consumption.

In study B, β -glucan enrichment of oatcakes that were consumed daily as a snack for six weeks by overweight and obese subjects did not influence daily energy intakes or result in energy or nutrient displacement when compared to a control cracker consumed for the same duration. There was also no effect on anthropometric measurements of weight, BMI, WC, SAD or body fat percentage. No effect was seen on fasted blood glucose or glycaemic

responses following an oral glucose load. There was no effect on BP or heart rate following test snack consumption.

The following conclusions can be made based on the results of the present PhD thesis:

1. Consumption of 4 g β -glucan acutely at breakfast did not impact on subsequent energy intakes in healthy normal-weight subjects.
2. 4 g of oat β -glucan consumed in a semi-solid breakfast significantly increased subjective feelings of satiety and fullness, however this did not translate into eating less at a subsequent meal or for the remainder of the day in healthy normal-weight subjects.
3. 4 g of oat β -glucan consumed in a semi-solid breakfast significantly blunted post-prandial glucose and insulin, with a significant decrease in GLP-1 at 90 minutes following test breakfast consumption.
4. Consumption of a β -glucan-enriched oatcake (4.46 g β -glucan) daily as a snack for six weeks had no effect on the daily energy intakes of overweight/obese healthy subjects.
5. No significant improvements were seen in markers of abdominal obesity (WC, SAD), body weight or body fat percentage after consuming β -glucan-enriched oatcakes daily for six weeks in overweight/obese subjects
6. No effects were seen in BP or glucose profiles of overweight/obese subjects who consumed β -glucan-enriched oatcakes daily for six weeks.

11.1 Future considerations

As mentioned, in recent years there has been an increased focus on understanding the satiating properties of foods and beverages and on developing approaches to communicate enhanced satiety as a functional benefit to an increasingly health-conscious consumer.

Claims on functional benefits such as enhanced satiety or “feeling fuller for longer” are tightly regulated and must be supported with robust data that demonstrate a sustained effect in human eating trials (Hetherington et al. 2013). EFSA (2011) concluded that a cause and effect relationship has not been established between the consumption of β -glucans from oats and barley and a sustained increase in satiety leading to a reduction in energy intake. Trials should therefore consider both the short and longer-term effects of consuming oat β -glucan to provide robust evidence for a possible EFSA health claim for an increase in satiety leading to a reduction in energy intake.

In this thesis, the effects of consuming 4 g oat β -glucan were assessed only in the acute appetite setting (<24 hours) in Study A and the longer-term effects of consuming a β -glucan-enriched snack containing 4.46 g β -glucan was investigated only in Study B, with both studies using different food vehicles to deliver oat β -glucan to the diet. It would therefore be advisable to assess the impact of consuming the test meal breakfast from Study A over a longer timeframe to identify how daily consumption may influence energy intakes, especially since favourable effects were seen in both acute glucose and insulin responses. Likewise, the short-term effects of consuming the oat β -glucan-enriched snacks from Study B should also be investigated to assess their impact on subsequent energy intake and glycaemic responses in an acute appetite setting. Performing such studies would provide valuable evidence to support the substantiation of health claims related to oat β -glucan and energy intakes. Additional parameters worthy of investigating the potential satiating nature of oat β -glucan are discussed in the following sections.

11.1.1 Gut fermentation

As awareness and understanding of the importance of the gut microbiome and gut microbiota increases, it is imperative for consumers to understand the key differences between different forms of prebiotics, and where they can be found in various foods and food products. Dietary fibre supplements are commonly consumed to help meet fibre recommendations and have

more recently become recognised as ‘prebiotics’ to improve GI health by stimulating beneficial bacteria and the production of SCFAs. It is estimated that SCFA production may contribute to up to 10% of the host’s metabolizable energy daily, with production of total SCFAs usually between 100–200 mmol/day, however this is highly dependent on the donor and availability of substrates for fermentation (Cook and Sellin 1998; Carlson et al. 2017). Although colonic fermentation was not a focus of the current study, it is important to acknowledge that by-products of colonic microbiota fermentation, propionate and butyrate, may play an influential role in satiety mechanisms (Alhabeeb et al. 2014; Byrne et al. 2016), particularly when it has been reported recently that Oatwell™ oat bran powder promotes propionate production. Five commonly consumed fibres, Oatwell™ oat bran powder, pure β -glucan, xylooligosaccharides (XOS), WholeFiber (dried chicory root containing inulin, pectin, and hemi/celluloses) and pure inulin were compared to assess fermentability, using an *in vitro* fermentation system measuring changes in faecal microbiota, total gas production and formation of common SCFAs in a recent study by Carlson et al. (2017). At 12 hours of fermentation, OatWell™ and pure β -glucan had significantly higher concentrations of propionate, and the highest mean concentration at 24 hours, compared to the other prebiotic dietary fibres investigated. A similar *in vitro* study with β -glucan based products has also shown similar preference for these products to result in propionate formation. When compared to inulin, butyrate production was also higher in terms of total amount and proportion of total SCFA produced (Hughes et al. 2008). Increases in circulating anorexigenic hormones, GLP-1, PYY (Tarini and Wolever 2010; Nilsson et al. 2013; Chambers et al. 2015) and decreases in circulating ghrelin (Parnell and Reimer 2009) are two potential mechanisms whereby SCFA may affect satiety. Nilsson et al. (2013) reported that feeding healthy participants an evening meal consisting of brown beans increases circulating PYY and decreases circulating ghrelin after a standard breakfast meal. This was attributed to propionate, as concentrations were significantly increased after the brown bean meal compared to the control. The study by Chambers et al. (2015) is the first study to show that increasing colonic propionate prevents

weight gain in overweight adult humans. Chambers et al. (2015) measured the release of GLP-1 and PYY from L-cells *in vitro* and found that SCFA led to significant increases in hormone release above basal levels. What is particularly innovative with regards to the work by Chambers and colleagues is that the group produced a novel ester which was able to bind propionate to inulin via an ester bond. This allowed delivery of propionate to the gut as inulin which was fermented by colonic fermentation, and as a result released propionate. In a 24-week follow-up supplementation study in 49 volunteers, 10 g of propionate per day led to significant reductions in energy intake by 162 kcal compared to the control group. This decrease was attributed to the increased stimulation of GLP-1 and PYY (Chambers et al. 2015). However, the results of these studies could also be due to other characteristics of fibre and not SCFA exclusively. Tarini and Wolever (2010) found increases in plasma GLP-1 concentrations and decreases in serum ghrelin following consumption of 24 g of inulin delivered via a test drink, effects of which are attributed to increased colonic SCFA production. Bearing in mind that the updated DRV for adults for fibre is 30 g per day, this is a large quantity of fibre to consume at a single meal. Nevertheless, it does prove the possibility for fermentable fibre to mediate satiety, potentially through increased SCFA levels from colonic microbiota fermentation.

Two orphan G-protein coupled receptors, GPR41 and GPR43, also known as free fatty acid receptors FFAR2 and FFAR3, are reported to be activated by SCFAs (Ang et al. 2018). Expression of FFAR2 and FFAR3 are highest in the distal small intestine (ileum) and (proximal) colon, in the large population of PYY and GLP-1 producing cells (Kaemmerer et al. 2010). Propionate has long been described as a hepatic gluconeogenic substrate (Anderson et al. 1984), however, De Vadder et al. (2014) have shown that propionate is converted into glucose by intestinal gluconeogenesis (IGN), i.e., in the intestine before it reaches the liver. Direct infusion of glucose into the hepatic portal vein (HPV) has been shown to have an inhibitory effect on eating (Langhans et al. 2001). Delivery of glucose into the HPV is detected within the lumen of which initiates the firing of a nervous signal to the nucleus of the solitary

tract and/or hypothalamus to inhibit eating (Mithieux 2009). Propionate and butyrate activate IGN via complementary mechanisms; butyrate activates IGN gene expression through a cAMP-dependent mechanism, while propionate, itself a substrate of IGN, does not directly stimulate IGN genes, but binds to FFAR3, which sends signals to the parabrachial and paraventricular nuclei in the brain (De Vadder et al. 2014). Furthermore, butyrate has been shown to increase directly expression of two regulatory enzymes involved in IGN, phosphoenolpyruvate carboxykinase 1 (PCK1) and glucose-6-phosphatase catalytic subunit (G6PC) in *in vitro* animal models (Hurst et al. 2014).

The satiating properties of propionate have also been attributed to reduce GER (Liljeberg and Björck 1996). In rats, colonic contractile activity has been shown to reduce following SCFA infusion to the colon (Squires et al. 1992), yet a more recent study showed no effect in humans (Jouët et al. 2013). However, Liljeberg and Björck (1996) reported that there were greater subjective satiety ratings linked to slower GE after SCFA ingestion. The literature surrounding SCFA is promising with regards to exerting beneficial effects on satiation, with more studies required to evaluate the overall benefits of β -glucan consumption from initial ingestion to colonic fermentation.

11.1.2 Glicentin

Glicentin, which is the largest molecule processed from proglucagon, is co-secreted from L-cells along with GLP-1, GLP-2, and oxyntomodulin (OXM). Like OXM, it possesses the sequence of glucagon. The biological actions of glicentin are reported to include insulin secretion, inhibition of gastric acid secretion, GE, and stimulation of mucosal enterocyte proliferation (Drucker et al. 2005; Pendharkar et al. 2017).

Due to its structure, assessment of circulating glicentin concentration is challenging to obtain a specific measurement which does not cross-react with other proglucagon-derived peptides, as glicentin peptide contains the entire sequence of glucagon, OXM and glicentin related

pancreatic polypeptide (GRPP). Lack of specificity in analytical techniques had led to the lack of commercialized methods to measure glicentin, thus literature on circulating glicentin in humans is poor and its variation is not fully understood. Yet more recently, commercialization of specific detection methods offers the opportunity to explore glicentin physiology, thus explore glicentin's potential involvement in appetite and eating.

To date there are no studies that have investigated the effect of β -glucan consumption on post-prandial glicentin and satiety outcome measures. However, there is evidence to suggest an effect of glicentin on GI motility in *in vitro* animal smooth muscle cells (Rodier et al. 1997; Jarrousse et al. 2004) and human enteric nerve responses (Tomita et al. 2005). In addition, several more recent *in vivo* studies have demonstrated that administration of glicentin led to an increase in plasma insulin and a decrease in plasma glucagon (Ohneda et al. 1986; Ohneda et al. 1988; Ohneda et al. 1995). Hence, in a similar fashion to GLP-1, glicentin has an insulintropic action and exerts incretin-like effects, thus may play a role in influencing appetite. As with other gut peptides, glicentin is stimulated by food ingestion, with reports of increased plasma glicentin concentrations following glucose, lipid and amino-acid loading into the duodenum of piglets (Ohneda et al. 1987; Ohneda 1987; Ohneda et al. 1988). However, for a gut peptide to exert physiological effects on eating, the efficacy of glicentin in amounts duplicating meal-related changes and administration of secretagogues for glicentin should be explored (Criteria 3 and 4, **Table 1, Chapter 2, section 2.4**). The problem with existing evidence is that firstly Ohneda and colleagues carried out the aforementioned studies around 30 years ago, as such sequencing to identify glicentin protein and technologies to identify and purify assaying have largely evolved, and secondly, there are no physiological ranges for human glicentin levels established. Now that adequate analytical techniques are available, further research should be undertaken to investigate the potential for glicentin to influence meal-related functions.

To develop a full picture of glicentin and its involvement in appetite, additional studies are required to investigate its role in meal-related functions. Emphasis should be on assessing

energy intake, along with relevant biomarkers of satiety, i.e., proglucagon family peptides, PYY and also CCK, to identify possible relationships between glicentin, satiety related gut peptides and physical eating, thus providing evidence for underpinning eating-inhibitory mechanisms. However, despite intestinal L-cell expression throughout the small bowel, addressing Criterion 2 in **Table 1 (Chapter 2, section 2.4)** may prove difficult since there is no known glicentin receptor identified. Therefore, identifying the role of glicentin in appetite physiology will be challenging to ascertain, as actions such as gut motility, stimulation of insulin and inhibition of gastric acid secretion are also ascribed to other proglucagon-derived peptides, such as GLP-1 and glucagon (Pendharkar et al. 2017). Nevertheless, exploring glicentin response in relation to consumption of β -glucan may offer explanation of satiating effects from an incretin-related mechanistic point of view.

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Appendices

Appendix 1: Summary of acute energy intake and appetite studies following cereal β -glucan consumption

Authors & β -glucan source	Study overview	Food intake test	Physiochemical measurements	Results summary	Conclusions
Beck et al. (2009a) & Beck et al. (2009b) <i>Oat</i>	Subjects: 14 overweight/obese Study design: crossover Intervention: five breakfast cereals, 0,2.16,3.82g,5.45 and 5.65g β -glucan Study duration: 5 visits separated by ≥ 3 days Subjective ratings: VAS 4-hr Plasma: ghrelin, CCK, glucose and insulin	IMI: 4-hr <i>Ad libitum</i> buffet lunch; sandwiches, dried fruit, nuts, yoghurt and juice	Concentration, solubility, viscosity and MW Viscosity at 30s ⁻¹ was reported and increased from 5.8 to 84.8 mPa.s as the β -glucan dose increased MW >10 ⁵ g/mol (high)	EI decreased following ≥ 5 g β -glucan by ~96kcal (vs. control) Subjective ratings: difference between all doses and control, but no dose response relationship Hormones: Dose response relationship between β -glucan and CCK. Correlation between β -glucan content and PYY release	β -glucan increases satiety possibly acting through CCK and PYY. Optimal dose of β -glucan apprx.4-6g
Clegg and Thondre (2014) <i>Barley</i>	Subjects: 23 healthy males, normal weight Study design: RCT Intervention: standardized breakfast followed by three soups; 0, 3.61 (LMW) and 12.88g (HMW) β -glucan Study duration: 3 days separated by ≥ 2 days	IMI: 2-hr <i>Ad libitum</i> sandwiches with various fillings, water	Viscosity of LMW and HMW soups at 37°C, shear rates 0.02 – 20s ⁻¹ LMW soup: 100Pa.s HMW soup: 350Pa.s	No eating-inhibitory effect at <i>ad libitum</i> lunch or EI the rest of the day. No differences in any of the VAS parameters between meals	Differences in physico-chemical properties of barley β -glucan cannot influence perceived feelings of hunger or actual EI

Subjective ratings: VAS before and after breakfast and soup, then every 30 min (t0-120)

Geliebter et al. (2015)	Subjects: 36 (18 lean/18 overweight) Study design: RCT Intervention: Breakfast; frosted corn flakes (<1 g fibre), quick cooking oatmeal (4 g soluble, 4 g insoluble fibre), and water only. Study duration: 3 mornings, separated ≥ 2 days Subjective ratings: VAS hunger and fullness (t0-180min) GER: acetaminophen Plasma: glucose, insulin, glucagon, leptin	IMI: 3-hr Liquid test meal (nutritionally complete formula)	N/A	Oatmeal reduced energy intake compared to frosted flakes or water. Overweight/obese subjects had lower energy intake than normal weight subjects Oatmeal increased fullness and reduced hunger compared to frosted flakes, and water Glucose area did not differ between the two cereals, nor did insulin AUC Oatmeal slowed GE compared to frosted flakes and water	Fullness and hunger ratings along with reduced <i>ad libitum</i> test meal intake may be related to slower GE following oatmeal
Hartvigsen et al. (2014)	Subjects: 15 with MetS Study design: RCT Intervention: wheat bread (0.2g), arabinoxylan bread (0.3g), β -glucan bread (4.2g) and rye kernel bread (1.5g β -glucan)	IMI: 4 ½ hr <i>Ad libitum</i> ham and cheese pizza, water	β -glucan content of breads measured HMW β -glucan extract used in breads (PromOat)	No eating-inhibitory effect of any breads Arabinoxylan, β -glucan and rye breads reduced hunger, and prospective consumption, and increased satiety. Only arabinoxylan	Breads with added fibre increase satiety with no effect on ghrelin concentrations, or EI

Study duration: 4 visits
separated by ≥ 1 week
Subjective ratings: VAS (t0-270min)
Plasma: GLP-1, GIP ghrelin

and rye breads increased fullness compared to wheat bread.

No differences in ghrelin.
GLP-1 was higher after rye kernel bread compared to arabinoxylan or β -glucan breads 120 - 270 minutes postprandially.
Rye kernel bread ingestion lowered the GIP AUC_{120min} compared to other breads

Hlebowicz et al. (2007)	Subjects: 12 healthy Study design: RCT Intervention: cornflakes, branflakes (0g) and wholemeal oatflakes (0.5g β -glucan) Study duration: 3 visits, separated ≥ 1 week Subjective ratings: 10pt scoring system (t0-120) GER: Ultrasonography at 15 and 90 mins postprandially Plasma: glucose	N/A	N/A	No differences in appetite ratings between meals N/S between branflakes and oat flakes compared to corn flakes branflakes had lower GER compared to oat flakes No differences in glucose responses between three meals	Fibre in semi-solid meal has no effect on satiety despite reduction in GER, and this is not influenced by blood glucose
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Hlebowicz et al. (2008)	Subjects: 12 normal weight Study design: cross-over Intervention: vanilla yoghurt, oatflakes and branflakes, 0g, 4g, 0g β -glucan, respectively. Study duration: 3 visits Subjective ratings: 10pt scoring system at 15, 90 mins postprandially GER: ultrasonography at 15, 90 min postprandially Plasma: Glucose (t0-60)			Attenuated glucose response after oatflakes compared to cornflakes at 30 min N/S GE between cornflakes and oatflakes N/S satiety between cornflakes and oatflakes	4g β -glucan in semisolid meal does not affect GE or satiety of healthy subjects compared to cornflakes. Reduction in blood glucose could not be explained by GE
<i>Oat</i>					
Juvonen et al. (2011)	Subjects: 20 normal weight Study design: cross-over Intervention: puddings with different insoluble and soluble fibre content; no fibre (0g β -glucan), wheatbran (0g β -glucan, 10.3g insoluble), oatbran (5.1g β -glucan), mixed fibre (2.5g β -glucan, 7.6g insoluble) Study duration: 4 visits, separated by ≥ 2 days Subjective ratings: VAS Plasma: glucose, insulin, ghrelin, PYY (t0-180)	IMI: 3-hr <i>Ad libitum</i> buffet-style lunch; vegetable soup, oat and rye breads, non-caloric orange juice, water Food records 24hr subsequent intake	N/A	No eating-inhibitory effect at <i>ad libitum</i> lunch or 24hr subsequent intake No effect on satiety or fullness between puddings Glucose lower following oatbran pudding compared to mixed fibre and no fibre pudding. Insulin lower following oatbran at 30,45,60 min compared to wheatbran. No effect on ghrelin and PYY	Consumption of different fibre types did not influence postprandial peptide release, appetite or subsequent EI, despite differences in insulin and glucose Food matrix may influence satiation nature of fibre.
<i>Oat</i>					

Juvonen et al. (2009) <i>Oat</i>	Subjects: 20 normal weight Study design: cross-over Intervention: two 300 mL isocaloric drinks; high viscosity drink and β -glucanase treatment to one drink to have low viscosity. Both drinks 5.1g β -glucan Study duration: 2 visits, separated ≥ 2 days Subjective ratings: VAS GER: paracetamol Plasma: glucose, insulin, GLP-1, CCK, PYY	Weighed food intake during study day <i>Ad libitum</i> lunch 3-h IMI	MW, viscosity Viscosity; Low viscosity drink <250 mPas High viscosity drink >3000 mPas, at 20 min	Low viscosity drink greater satiating effect High viscosity drink more filling. N/A on EI GE faster following low viscosity drink Greater insulin, CCK, PYY, GLP-1 after low viscosity drink	Lowering natural viscosity of oat β -glucan attenuates glucose and insulin and accelerates GE. Appetite and EI are not affected despite a clear difference in viscosity or enhances responses from GI tract
Korczak et al. (2014) <i>Oat and barley</i>	Subjects: 42 healthy, normal weight Study design: RCT Intervention: three breakfast bars with different fibre content; 10 g oat bran, 10 g barley bran, 3 g dietary fibre Study duration: 3 visits, separated by ≥ 1 week Subjective ratings: VAS (t0-240) Colonic fermentation: breath test at 0 and 240min	IMI: 4-hr <i>Ad libitum</i> lunch; pizza, water Food record for remainder of study day	N/A	No differences in EI at <i>ad libitum</i> meal or subsequent EI No differences in appetite ratings No differences in hydrogen or methane production	Oat bran does not induce greater satiety than barley bran or a low fibre control

Lyly et al. (2009)	Subjects: 19 normal weight Study design: crossover Intervention: three drinks with three different fibres; guar gum, wheatbran and β -glucan, wheatbread (control), drink w/o fibre (control) Study duration: 5 visits, separated by ≥ 1 day Subjective ratings: VAS (t0-120)	N/A	Viscosity: Drink without fibre 1.7 mPas Wheat bran drink 2.6 mPas, Oat β -glucan drink 33.5 mPas Viscosity of the oat β -glucan drinks increased to 200 mPas after 20 min from preparation of the sample	AUC for perceived satiety was higher and the desire to eat was lower for the guar gum drink compared to the drink without fibre. Oat β -glucan drink increased fullness and showed a trend of increasing perceived satiety and decreasing the desire to eat more than the drink without fibre.	Dietary fibre in beverages can enhance their perceived satiety and decrease the desire to eat more than a beverage without fibre
Lyly et al. (2010)	Subjects: 29 normal weight Study design: crossover Intervention: two drinks without fibre energy content 167kcal and 335kcal; two drinks containing 2.5 and 5 g oat β -glucan (both 167kcal), one drink containing 5 g oat β -glucan (335kcal) and one drink containing 5g enzymatically treated oat β -glucan with low	N/A	Viscosity no fibre drink 1.5 mPas enzyme treated 31.6 mPas 5g β -glucan 661 mPas	Drink containing 2.5 or 5 g of β -glucan had a larger AUC for satiety and smaller AUC for hunger compared to the drink without fibre, but was not dose-responsive Energy content did not influence satiety low-viscosity β -glucan	β -glucan increases viscosity and enhances satiety but the effect may not be related to the amount of fibre

	viscosity at energy level 167kcal Study duration: 6 visits, separated by ≥ 1 day Subjective ratings: VAS (t0- 180)			increased satiety compared to no fibre containing drink, but less compared to the drink with the same amount of fibre and higher viscosity.	
Pentikäinen et al. (2014) <i>Oat</i>	Subjects: 30 normal weight Study design: RCT Intervention: two types of biscuits (0g and 4g oat β - glucan), two types of juice (0g and 4g β -glucan). Combinations, 0g, 4g, 4g, 8g β - glucan Study duration: 4 visits, separated by ≥ 2 days Subjective ratings: VAS (t0- 210)	N/A	Viscosities measured in artificial digestion conditions (gastric – intestine) Enriched biscuits + juice 4 g β -glucan: 2920 - 8160 mPa.s Biscuits + enriched juice 4 g β -glucan: 2100 - 6290 mPa.s Both enriched 8 g β - glucan : 19890 - 22730 mPa.s	Increased satiety with β -glucan-enriched biscuits and juice (8 g) compared to 0, or 4 g β - glucan. β -glucan in juice had a greater impact on satiety than when added to biscuits	4g or 8 g β - glucan increases satiety. Food matrix is an important factor influencing satiety
Peters et al. (2009)	Subjects: 21 healthy overweight Study design: 4-way balanced order crossover	IMI: 4-hr <i>Ad libitum</i> single item lunch;	Viscosity, gastric the addition of β - glucan to the bar	The addition of β -glucan, FOS, or a combination of FOS and β -glucan also did	β -glucan, FOS, or a combination meal replacement bars did not diminish hunger or

<i>Barley</i>	Intervention: meal-replacement bar; control bar (0.3g β -glucan), barley bar (1.2g β -glucan, 1.2g FOS), FOS bar (0.3g β -glucan, 8g FOS), FOS+barley bar (1.2g β -glucan, 8g FOS) Study duration: 4 visits, separated by 1 week Subjective ratings: self-assessment EVAS over 11-hr	macaroni and turkey	greatly increased in vitro gastric viscosity. β -glucan generated a doubling of in vitro gastric viscosity; Control bar 351 mPas β -glucan 841 mPas	not significantly affect food intake or subjective appetite	have an eating-inhibitory effect
Rebello et al. (2013) <i>Oat</i>	Subjects: 48 healthy overweight Study design: RCT Intervention: oatmeal (2.63g β -glucan) or RTEC (1.73g β -glucan) Study duration: 2 visits, separated by ≥ 1 week Subjective ratings: EVAS (t0-240)	N/A	β -glucan content, MW, gastric viscosity, radius of gyration MW: Oatmeal 3.78×10^5 RTEC 2.21×10^5 Da Over 2-hrs, viscosity of oatmeal (173-73mPas) remained more viscous than RTEC (1063-84mPas) Radius of gyration; β -glucan molecules in oatmeal (49.20 ± 2.56 nm) was greater than RTEC (36.83 ± 0.41 nm)	Greater increase in fullness, greater reduction in hunger, desire to eat and prospective food consumption following oatmeal Oatmeal had greater β -glucan content, higher viscosity, MW and hydration spheres compared to RTEC	Oatmeal improves appetite control and increases satiety. The effects may be attributed to the viscosity and hydration properties of its β -glucan content.

Rebello et al. (2014)	Subjects: 48 healthy mixed weights Study design: RCT Intervention: Old fashioned oatmeal (SO) and instant oatmeal (IO; 1.6 g β -glucan), oat-based RTEC (1 g β -glucan) Study duration: 3 visits separated by ≥ 1 week Subjective ratings: EVAS (t0-240)	N/A	β -glucan content, MW, gastric viscosity, radius of gyration MW: SO oatmeal: 3.89×10^5 Da IO: 3.78×10^5 Da RTEC: 2.21×10^5 Da Over 2hrs viscosity: IO: 7397-88mPas SO: 1063-85 mPas RTEC: 175-76 mPas Radius of gyration; β -glucan molecules in SO (48.2 nm) and IO (50.23 nm) was greater than RTEC (36.83 nm)	IO reduced desire to eat compared to RTEC Prospective intake was lower following SO and IO compared to RTEC	Oatmeal increases satiety compared to RTEC. Initial viscosity may be important for inducing satiety
Rebello et al. (2016a)	Subjects: 48 healthy mixed weights Study design: RCT	IMI: 4-hr	β -glucan content, MW, gastric viscosity, radius of gyration	Reduction of 85kcal at <i>ad libitum</i> lunch following oatmeal breakfast compared to RTEC	Energy intake was reduced following consumption of instant

<i>Oat</i>	<p>Intervention: oatmeal (2.68g β-glucan) and RTEC (1.73g β-glucan)</p> <p>Study duration: 2 visits, separated by ≥ 1 week</p> <p>Subjective ratings: EVAS (t0-240)</p>	<p><i>Ad libitum</i> lunch; turkey, ham, roast beef, or vegetable patty sandwiches, calorie-free or calorie-containing beverage.</p>	<p>MW: Oatmeal 3.89×10^5 RTEC 2.21×10^5 Da</p> <p>Viscosity; Oatmeal 7220 mPas RTEC 140 mPas</p> <p>Radius of gyration; β-glucan molecules in oatmeal (50.23 nm) was greater than RTEC (36.83 nm)</p>	<p>Increased fullness and reduced hunger following oatmeal breakfast compared to RTEC</p>	<p>oatmeal compared to the RTEC</p> <p>Oatmeal suppresses appetite, increases satiety, and reduces subsequent energy intake</p>
<p>Vitaglione et al. (2009)</p> <p><i>Barley</i></p>	<p>Subjects: 14 healthy normal weight</p> <p>Study design: Crossover</p> <p>Intervention: control bread (0g β-glucan) and β-glucan-enriched bread (3g)</p> <p>Study duration: 4 visits; 2 for blood sampling, 2 for appetite and EI, separated by ≥ 1 week</p> <p>Subjective ratings: VAS (t0-180)</p> <p>Plasma: glucose, insulin, PYY</p>	<p>IMI: 3-hr</p> <p><i>Ad libitum</i> lunch: Italian-style tomato pasta, cold rice salad, fish, meat, green salad, chips, bread, fruit</p>	<p>N/A</p>	<p>Reduction of 172 kcal at <i>ad libitum</i> lunch following β-glucan-enriched bread compared to control bread</p> <p>Reduction in hunger and increased fullness following β-glucan-enriched bread</p> <p>Higher AUC for PYY following β-glucan-enriched bread</p>	<p>Barley β-glucans were able to control appetite in the short term by modulating sensations and reducing EI</p>

				AUC ₆₀₋₁₈₀ for ghrelin was lower following β -glucan-enriched bread	
				No effect on insulin but reductions in postprandial glucose	
Vitaglione et al. (2010)	Subjects: 20 healthy normal weight Study design: crossover Intervention: fixed standard breakfast followed by midmorning snacks; small and large preload β -glucan biscuits, small and large control biscuit snacks. Study duration: 5 visits, separated by 1 week Subjective ratings: VAS (t0-120)	IMI: 2-hr	N/A	No eating-inhibitory effect identified. Increase of total food intake in men after midmorning snack and in women after the large preload Decrease AUC desire to eat and an increase AUC of fullness and satiety were recorded with small preload β -glucan biscuits compared to small preload control biscuits	β -glucan biscuits consumed as a midmorning snack, although able to influence appetite ratings, did not modify food intake in a short time period
Willis et al. (2009)	Subjects: 20 healthy normal weight Study design: RCT	N/A	N/A	Cornbran and resistant starch muffins were more	Not all fibres effect satiety equally

<i>Barley</i>	<p>Intervention: low fibre muffin vs. 4 high fibre muffins; cornbran (9.1g insoluble fibre), barley (4g β-glucan), resistant starch (7.9g insoluble fibre), polydextrose (8g)</p> <p>Study duration: 5 visits, separated by ≥ 1 week</p> <p>Subjective ratings: VAS (t0-180)</p>	<p>satiating than low fibre muffin.</p> <p>Cornbran and resistant starch muffins influenced duration of satiety longer than other muffins</p>
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AUC, area under curve; *CCK*, cholecystokinin; *Da*, Dalton; *EI*, energy intake; *EVAS*, electronic visual analogue scale; *FOS*, fructo-oligosaccharide; *g*, grams; *GE*, gastric emptying; *GIP*, gastric inhibitory polypeptide; *GLP-1*, glucagon-like peptide 1; *hr*, hour; *IO*, instant oats; *kcal*, calorie; *MetS*, metabolic syndrome; *mPas*, millipascal second; *MW*, molecular weight; *N/S*, no significance; *nm*, nanometer; *Pas*, pascal second; *PYY*, peptide YY; *RCT*, randomised controlled trial; *RTEC*, ready-to-eat cereal; *s⁻¹*, per second; *SO*, old-fashioned oats; *t*, time; *VAS*, visual analogue scale

Appendix 2: Participant Information Sheet Study A

Information Sheet

My name is Suzanne Zaremba and I am a PhD student undertaking my research in the school of Dietetics, Nutrition and Biological Sciences at Queen Margaret University, Edinburgh.

The title of my project is: "***Oat β -glucan, perceived satiety and ad libitum food intakes.***"

This study will investigate what impact, if any, eating soluble fibre (β -glucan) at breakfast will have on appetite for the rest of the day. I want to see if eating β -glucan will influence the release of gut hormones which contribute to feelings of hunger and fullness.

Who am I looking for?

I am looking for 33 or more volunteers to participate in this short term study. Unfortunately, there are inclusion and exclusion criteria which will determine your participation in the study. To be eligible to take part you must be 18-50 years old, have a body mass index of 20.0 to 29.9kg/m² and be in general good health. Smokers and individuals who suffer from (or taking medication for) cardiovascular or gastro-intestinal disease are not eligible for participation. Anyone who presents with signs of anemia or impaired glucose tolerance will be exempt (I will check these measurements during screening). Females who are pregnant or breastfeeding, or are postmenopausal are also excluded.

What am I required to do?

If you agree to participate in the study, you will be asked to come along to three consultations. Please note, in order to measure gastrointestinal hormones you will be required to have a number of blood samples taken. You will have an intravenous cannula inserted into your arm in order to facilitate serial blood sampling. Therefore if you have anxiety or needle phobia it is not recommended you participate. In addition, if you have any food allergies or dislikes to the ingredients listed below you will also be exempt from participation.

Screening Visit

On the first visit you will be screened to check your eligibility for participation. You will have your height, weight, fasting blood glucose and haemoglobin measured. Fasting blood glucose and haemoglobin measurements will involve a finger-prick blood sample being taken. This will require two 'drops' of blood from your fingertip. You will also be given three short questionnaires to complete. If you are eligible and want to continue with the study, you will be required to complete a 3 day food diary before beginning the study. The screening visit will last around 45-60 minutes.

Test days

On the second and third visit, you will be required to return your completed 3 day food diary and arrive at the research centre having fasted for at least 8 hours from both solids and liquids. Before each visit, you will be instructed to abstain from caffeine,

alcohol and strenuous exercise for 24 hours. You will be given a standard breakfast to eat, consisting of Rice Krispies, semi-skimmed milk and Greek style yoghurt. On visits 2 and 3 you will receive a breakfast either with or without added fibre. Powder will be added to breakfast meals in order to increase the fibre content of the meal.

Your appetite will be measured before breakfast and then recorded over a 2.5 hour period afterward, where you will be asked to complete simple hunger rating scales and have blood samples taken. The total amount of blood taken from finger-prick tests will be 4.8µL, which equates to less than half a teaspoon in volume and a total of 40mL of blood will be withdrawn from the intravenous cannula (less than 3 tablespoons). You will then be given a lunch to consume, which will contain white bread, butter and ham. You will then be asked to record your diet for the remainder of the day and day after. Visits 2 and 3 will last around 3.5 hours each day.

What are the possible risks of taking part?

We are not aware of any harmful risks associated with any of the methods used to carry out the study. However it is important to point out that consumption of fibre may result in altered gastrointestinal-comfort such as bloating or flatulence, yet the likelihood of this occurring is low.

During blood sampling you may feel uncomfortable for a few seconds when the intravenous cannula is inserted into your vein and may bleed a little when the cannula is removed. Pressure will be applied by holding the puncture site firmly for a few minutes. Immediately following the removal of the cannula you may feel a little dizzy; this is not unusual and can be minimized by resting.

There may be a possibility of bruising around the puncture site following blood sampling, which can be reduced by applying digital pressure for around 2 minutes once the cannula has been removed. It is advised that you avoid bending your arm to prevent subsequent bleeding/bruising. The smallest possible sized cannula will be used in order to reduce possible discomfort. The researcher will ensure that high standards of practice are maintained throughout the session and termination of the blood sampling procedure will occur should you show any signs of distress.

You will be free to withdraw from the study at any stage without giving reason.

This research study is financially sponsored by DSM, a science-based company active in health and nutrition. Data from the study will be shared with DSM, and additionally may be published in a journal or presented at a conference. All data collected will be anonymized and no personal identifiable information will be disclosed to the sponsor. Your name will be replaced with a participant number, and it will not be possible for you to be identified in any reporting of the data gathered.

Your information will be entirely anonymous; it will be scanned and stored in a password protected computer file that only the researcher will have access to. Blood samples will be stored anonymously in the research laboratory for up to 5 years in case of any necessary repeat analysis. Samples will not be used for any other research purposes, only for the purpose of this research study. After 5 years all information will be destroyed.

Please note: It is not uncommon for research to incidentally discover signs of disease or abnormality. If any abnormal results are found (for example impaired glucose tolerance, anaemia or high blood pressure) you will be advised by the researcher to seek advice from appropriate health care professionals.

If you would like to contact an independent person, who knows about this project but is not directly involved in it, you are welcome to contact Dr. Douglas McBean. His contact details are given below.

If you have read and understood this information sheet, any questions you had have been answered, and you would like to be a participant in the study, please now see the consent form.

Contact details of the researcher

Name of researcher: Suzanne Zaremba

Address: School of Health Sciences,
Department of Dietetics, Nutrition and Biological Sciences,
Queen Margaret University
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Email: SZaremba@qmu.ac.uk

Telephone: 0131 474 0000 – say 'Suzanne Zaremba' when prompted by our automated system

Contact details of the independent adviser

Name of adviser: Dr. Douglas McBean

Address: Senior Lecturer in Physiology & Neuroscience
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Telephone: 0131 474 0000 – say 'Douglas McBean' when prompted by our automated system

Thank you for your time and consideration.

Appendix 3: Dutch Eating Behaviour Questionnaire

The Dutch Eating Behaviour Questionnaire

Eating Behaviour Questionnaire

Please answer all the questions below. Please tick the box which most closely represents your normal behaviour.

	Never	Seldom	Sometimes	Often	Very often
If you have put on weight do you eat less than you usually do?					
Do you try to eat less at mealtimes than you would like to eat?					
How often do you refuse food or drink offered because you are concerned about your weight?					
Do you watch exactly what you eat?					
Do you deliberately eat foods that are slimming?					
When you have eaten too much do you eat less than usual the following days?					
Do you deliberately eat less in order not to become heavier?					
How often do you try not to eat between meals because you are watching your weight?					
How often in the evening do you try not to eat because you are watching your weight?					
Do you take into account your weight with what you eat?					
	Never	Seldom	Sometimes	Often	Very often
Do you have the desire to eat when you are irritated?					
Do you have a desire to eat when you have nothing to do?					
Do you have a desire to eat when you are depressed or discouraged?					
Do you have a desire to eat when you are feeling lonely?					
Do you have a desire to eat when somebody lets you down?					
Do you have a desire to eat when you are cross?					
Do you have a desire to eat when you are approaching something unpleasant to happen?					
Do you get the desire to eat when you are anxious, worried or tense?					
Do you have a desire to eat when things are going against you or when things have gone wrong?					
Do you have a desire to eat when you are frightened?					

Do you have a desire to eat when you are disappointed?					
Do you have a desire to eat when you are emotionally upset?					
Do you have a desire to eat when you are bored or restless?					
	Never	Seldom	Sometimes	Often	Very often
If food tastes good to you do you eat more than usual?					
If food smells and looks good, do you eat more than usual?					
If you see or smell something delicious do you have a desire to eat it?					
If you have something delicious to eat, do you eat it straight away?					
If you walk past the baker do you have the desire to buy something delicious?					
If you walk past a snack bar or café do you have the desire to buy something delicious?					
If you see others eating do you also have the desire to eat?					
Can you resist eating delicious foods?					
Do you eat more than usual, when you see others eating?					
When preparing a meal are you inclined to eat something?					

Appendix 4: Scottish Physical Activity Questionnaire

<p>The following questions relate to your physical activity over the previous week. Please mark in the appropriate box the number of minutes spent doing a particular activity. Please try and think carefully and be as accurate as possible with your answers and only include activities of either moderate or vigorous intensity. Examples are given of what should and should not be included.</p>	✖	LIGHT INTENSITY - Your heart rate and breathing rate are no different from what they are when you are standing, sitting etc
	✓	MODERATE INTENSITY - Your heart rate and breathing rate are faster than normal. You may also sweat a little. Brisk walking or sweeping and mopping are good examples of how you might feel
	✓	VIGOROUS INTENSITY - Your heart rate is much faster and you have to breathe deeper and faster than normal. You will probably sweat. Playing football or squash are good examples of how you might feel

LEISURE TIME PHYSICAL ACTIVITY - Remember, **do not** include light intensity activities

In the past week how many minutes did you spend each day:	MON	TUES	WED	THUR	FRI	SAT	SUN	TOTAL
Walking outwith work?								
DO include ✓ e.g. walking to the shops, walking to work, walking the dog, stairwalking ✓								
DO NOT include ✖ e.g. standing, sitting, driving, walking whilst at work ✖								
Manual labour outwith work?								
DO include ✓ e.g. cutting grass, decorating, washing car, DIY, digging ✓								
DO NOT include ✖ e.g. weeding, planting, pruning ✖								
Active housework?								
DO include ✓ e.g. vacuuming, scrubbing floors, bed making, hanging out washing ✓								
DO NOT include ✖ e.g. sewing, dusting, washing dishes, preparing food ✖								
Dancing?								
DO include ✓ e.g. only include time actually spent dancing ; disco, line, country ✓								
DO NOT include ✖ e.g. time spent not actually dancing ✖								
Participating in a sport, leisure activity or training?								
DO include ✓ e.g. exercise classess, cycling, football, swimming, golf, jogging, athletics ✓								
DO NOT include ✖ e.g. darts, snooker / pool, fishing, playing a musical instrument ✖								
Other Physical Activity if not already covered (please write in)								
								TOTAL

PHYSICAL ACTIVITY AT WORK (Only complete if you are currently employed and remember not to include light intensity activities)

In the past week how many minutes did you spend each day:	MON	TUES	WED	THUR	FRI	SAT	SUN	TOTAL
Walking whilst at work?								
DO include ✓ e.g. walking up or down stairs, to and from your desk, "doing the rounds" ✓								
DO NOT include ✖ e.g. standing, sitting at desk etc; i.e. time spent not actually walking ✖								
Manual labour whilst at work?								
DO include ✓ e.g. lifting, stacking shelves, climbing ladders, building work, cleaning ✓								
DO NOT include ✖ e.g. sitting at desk, answering telephone, driving, check-out operation ✖								
								TOTAL

Was last week typical of the amount of physical activity you usually do?

YES				
NO - I usually do more		Normally, how much more?		Of which activity?
NO - I usually do less		Normally, how much less?		Of which activity?

Appendix 5: Visual analogue scale

Visual Analogue Scale

Participant Number _____

Visit _____

Time _____

Please indicate using an **X** on the line below that best describes:

How **HUNGRY** do you feel *at this moment*?

Not hungry at all _____ Extremely hungry

How **FULL** do you feel *at this moment*?

Not full at all _____ Extremely full

How **STRONG** is your desire to eat *at this moment*?

Not strong at all _____ Extremely

How **SATIATED** are you *at this moment*?

Not at all _____ Extremely

How **MUCH** do you think you could (or would want to) eat *right now*?

Nothing at all _____ A very large amount

Please indicate using an **X** on the line below that best describes:

Visual appeal

Bad  Good

Smell

Bad  Good

Taste

Bad  Good

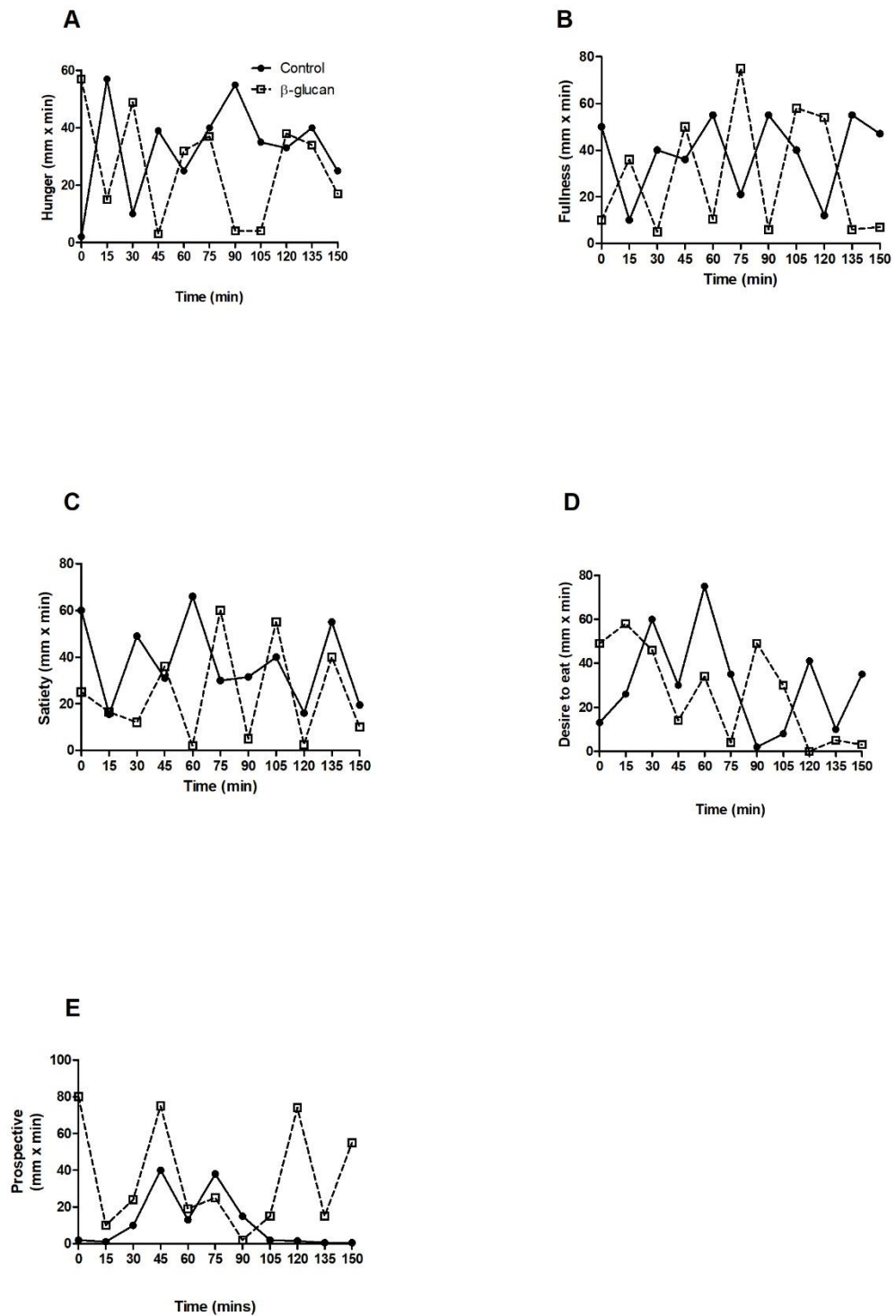
Aftertaste

Much  None

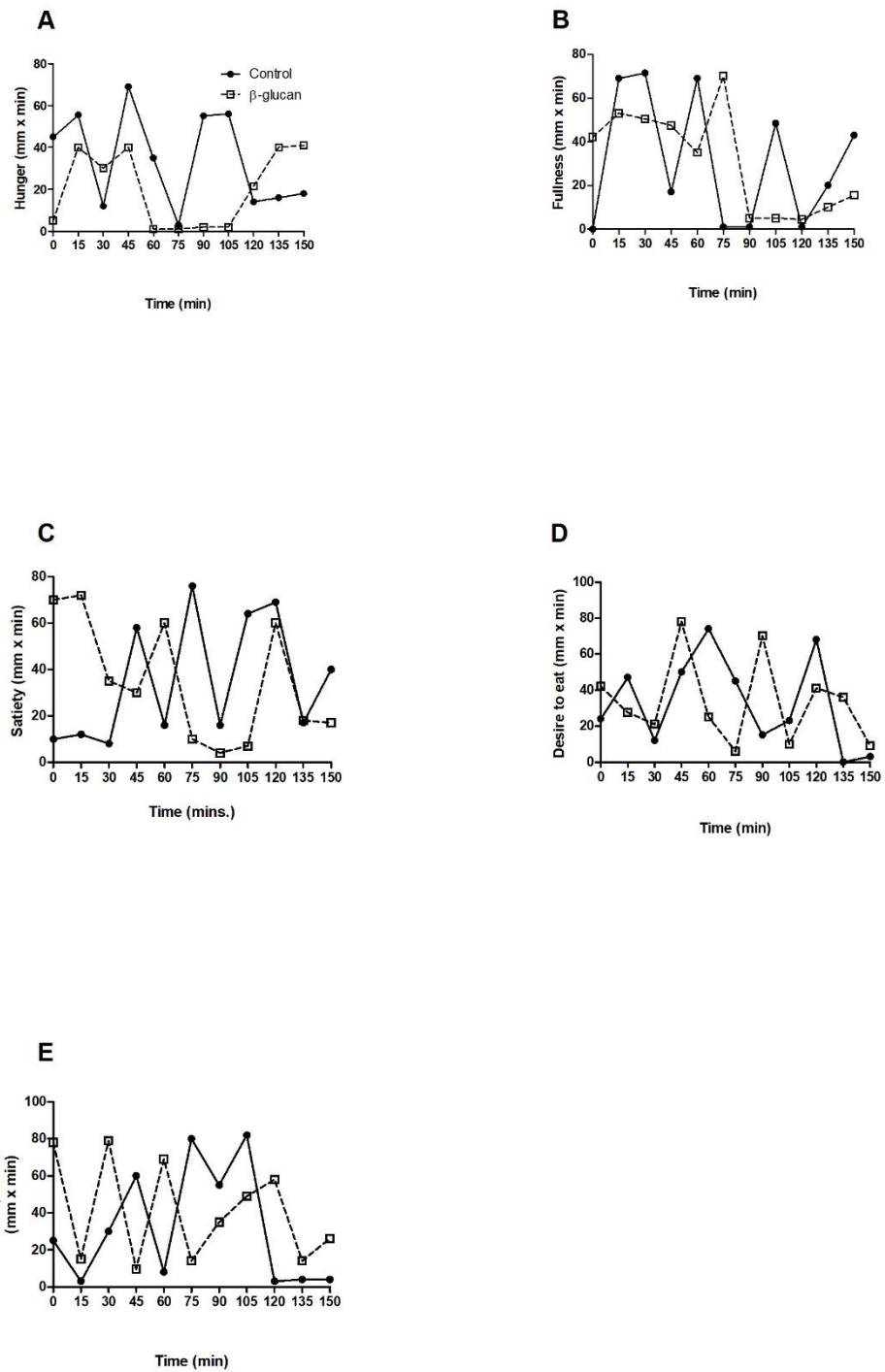
Palatability

Bad  Good

Appendix 6: Subjects 201 & 204



Visual analogue scale responses from subject **201** for subjective hunger (A), fullness (B), satiety (C), desire to eat (D) and prospective food consumption (E).



Visual analogue scale responses from subject **204** for subjective hunger (A), fullness (B), satiety (C), desire to eat (D) and prospective food consumption (E).

Appendix 7: Participant Information Sheet Study B



Queen Margaret University

EDINBURGH

Information Sheet

My name is Suzanne Zaremba and I am a PhD student undertaking my research in the school of Dietetics, Nutrition and Biological Sciences.

The title of my project is: *“Investigating the effects of consuming fibre on physiological parameters involved in the development of metabolic disease.”*

This study will investigate what impact, if any, eating soluble fibre on a daily basis will have on body composition and parameters which may predispose the development of metabolic disease.

Who am I looking for?

I am looking for up to 90 or more volunteers to participate in this dietary intervention study. Unfortunately, there are inclusion and exclusion criteria which will determine your participation in the study. To be eligible to take part you must be 18 years old or older and have the following criteria

- Be in general good health
- Males with waist circumferences of 94cm (37 inches) or more
- Females with waist circumferences of 80cm (31.5 inches) or more

OR Males and females with a body mass index (BMI) of 30kg/m² or more

Smokers and individuals who suffer from (or taking medication for) cardiovascular or gastro-intestinal disease are not eligible for participation. Females who are pregnant or breastfeeding, or are postmenopausal are also excluded.

What am I required to do?

If you agree to participate in the study, you will be asked to come along to three consultations. You will be required to eat a savoury snack provided free by the researcher every day for 6 weeks and agree to have a number of measurements taken, which are listed below.

Please note, you will be required to have blood samples taken (5 finger-prick samples) therefore if you have anxiety or needle phobia it is not recommended you participate.

Screening Visit

On the first visit you will be screened to check your eligibility for participation. You will have anthropometric measurements taken and the researcher will test your fasting blood glucose levels by taking a finger-prick sample of blood around 50µL in volume (one “drop” of blood). You will also be given three short questionnaires to complete. If you are eligible and want to continue with the study, you will then be required to complete a 7 day food diary before beginning the study. The screening visit will last around 45-60 minutes.

Test days

On the second visit you will return your completed food diary have the following measurements taken:

- Waist circumference, height and weight
- Sagittal abdominal diameter - an abdominal caliper will be used to measure central adiposity
- Bioelectrical Impedance Analysis - Electrodes will be placed on your wrist, hand, ankle and foot. Leads will be attached to electrodes and a Bodystat Analyser will calculate the body's resistance and reactance to the electrical current which determines your fat and lean (muscle) mass
- Blood pressure – a blood pressure monitor will measure your systolic and diastolic blood pressure
- Blood sampling – finger-prick blood samples will be taken to determine your response to glucose (sugar).

After measurements have been taken, the researcher will provide you with a savoury snack to consume on a daily basis for 6 weeks.

You will be asked to complete food diaries after 3 weeks of consuming the savoury snack and again during the last week before returning to the research centre for the final time. All tests that were carried out during the second visit will be performed again. Visits 2 and 3 will last around 2 hours each day.

What are the possible risks of taking part?

We are not aware of any harmful risks associated with any of the methods used to carry out the study. However, it is important to point out that consumption of fibre may result in altered gastrointestinal discomfort such as bloating or flatulence, yet the likelihood of this occurring is low.

During blood sampling you may feel uncomfortable for a few seconds. Pressure will be applied by holding the puncture site firmly for a few minutes. There may be a possibility of bruising around the puncture site following fingerpricks, which can be reduced by applying digital pressure for around 2 minutes once the sample has been taken. The researcher will ensure that high standards of practice are maintained throughout the session and termination of the blood sampling procedure will occur should you show any signs of distress.

Please note: It is not uncommon for research to incidentally discover abnormalities (apparent high/low levels beyond the normal range for the given parameter being tested). If any abnormal results are found, for example impaired glucose tolerance, or high blood pressure, you will be advised by the researcher to seek advice from appropriate health care professionals.

You will be free to withdraw from the study at any stage without giving reason.

This research study is financially sponsored by Nairn's Oatcakes limited. Data from the study will be shared with Nairn's, and additionally may be published in a journal or presented at a conference. All data collected will be anonymized and no personal identifiable information will be disclosed to the sponsor. Your name will be replaced with a participant number, and it will not be possible for you to be identified in any reporting of the data gathered.

If you would like to contact an independent person, who knows about this project but is not directly involved in it, you are welcome to contact Dr. Douglas McBean. His contact details are given below.

If you have read and understood this information sheet, any questions you had have been answered, and you would like to be a participant in the study, please now see the consent form.

Contact details of the researcher

Name of researcher: Suzanne Zaremba

Address: School of Health Sciences,
Department of Dietetics, Nutrition and Biological Sciences,
Queen Margaret University
Queen Margaret University Drive
Musselburgh
East Lothian
EH21 6UU

Email: SZaremba@qmu.ac.uk

Telephone: 0131 474 0000 – say 'Suzanne Zaremba' when prompted by our automated system

Contact details of the independent adviser

Name of adviser: Dr. Douglas McBean

Address: Senior Lecturer in Physiology & Neuroscience

School of Health Sciences
Queen Margaret University
Queen Margaret University Drive
Musselburgh
EH21 6UU

Email: DMcbean@qmu.ac.uk
Telephone: 0131 474 0000 – say 'Douglas McBean' when prompted by
our automated system

Thank you for your time and consideration.

Appendix 8: Examples of Under- and Overreporting calculations using Goldberg-Black equations

Before evaluating EI_{rep} in the current study, a number of factors were calculated for all subjects:

- BMRest (Schofield 1985)
- EI_{rep} (average of 7-day food intake)
- EI_{rep}:BMRest

In order to proceed with evaluating EI_{rep}, the appropriate PAL were taken from EFSA (2013) Panel in the scientific opinion for DRV for energy. Note, all subjects in the current study were in the age category of 18-69 years. Therefore, the following PAL were determined for each subject based on their activity levels:

Low 1.4 Moderate 1.6 Vigorous 1.8

Calculation of the variation in energy intake (S), BMR and PAL is required for the Goldberg/Black equation. S can be given by the equation:

$$S = \sqrt{((CV_{wEI}^2)/d) + CV_{wB}^2 + CV_{tP}^2}$$

Where:

CV_{wEI} is the within-subject variation in energy intake

d is the number of days of dietary assessment

CV_{wB} is the within-subject variation in repeated BMR measurements or the precision of estimated BMRest compared with measured BMR. This includes both measurement error and variation with time on repeated measurements

CV_{tP} is the total (between-subject) variation in PAL, but this figure also within-subject variation and methodological errors (Black 2000)

SD_{min} is -2 for the 95% lower confidence limit

SD_{max} is 2 for the 95% upper confidence limit

The revised factors by Black (2000) were applied to the equation for S, with the diet diary period for the current study, d = 7.

$$\begin{aligned} S &= \sqrt{((23^2 / 7) + 8.5^2 + 15^2)} \\ &= \sqrt{(75.6 + 72.25 + 225)} \\ &= \sqrt{(372.85)} = \mathbf{19.3} \end{aligned}$$

EXAMPLE OF MISREPORTING AT GROUP LEVEL

Intervention group (n=11), with low physical activity (PAL =1.4) and mean Elrep:BMRest of 1.12

$$\begin{aligned}\text{Lower cut off} &= \text{PAL} \times \exp [\text{SDmin} \times ((S/100)/ \sqrt{n})] \\ &= 1.4 \times \exp [-2 \times ((19.3/100)/ \sqrt{11})] \\ &= 1.246\end{aligned}$$

$$\begin{aligned}\text{Upper cut off} &= \text{PAL} \times \exp [\text{SDmax} \times ((S/100)/ \sqrt{n})] \\ &= 1.4 \times \exp [2 \times ((19.3/100)/ \sqrt{11})] \\ &= 1.573\end{aligned}$$

The calculated mean of the Elrep:BMRest for the group (1.12) is lower than the calculated specific lower cut-off (1.246) which means overall bias of underreporting in the studied group.

EXAMPLE OF MISREPORTING AT INDIVIDUAL LEVEL

Subject 107 had a moderate PAL (1.6) and EIrep:BMRest of 0.67, n=1.

$$\begin{aligned}\text{Lower cut off} &= \text{PAL} \times \exp [\text{SDmin} \times ((S/100)/ \sqrt{n})] \\ &= 1.6 \times \exp [-2 \times ((19.3/100)/ \sqrt{1})] \\ &= 1.09\end{aligned}$$

$$\begin{aligned}\text{Upper cut off} &= \text{PAL} \times \exp [\text{SDmin} \times ((S/100)/ \sqrt{n})] \\ &= 1.6 \times \exp [2 \times ((19.3/100)/ \sqrt{1})] \\ &= 2.35\end{aligned}$$

The EIrep:BMRest for subject 107 (0.67) is lower than the calculated specific lower cut-off (1.09) which means overall bias of underreporting in this subject.

Appendix 9: Sensory Evaluation Questionnaire of Study Snacks

Sensory Questions

Participant _____

With regards to the snack that you have eaten over the past 6 weeks, please rank the snack from 1 (extremely dislike) to 7 (extremely like) for the following attributes.

Please also write down any additional information that you think is relevant in the box below.

	←dislike					like→	
Appearance (colour)	1	2	3	4	5	6	7
Aroma	1	2	3	4	5	6	7
Taste	1	2	3	4	5	6	7
Texture (crunchiness)	1	2	3	4	5	6	7
Aftertaste (length of flavour)	1	2	3	4	5	6	7
Overall Liking	1	2	3	4	5	6	7

Additional comments: